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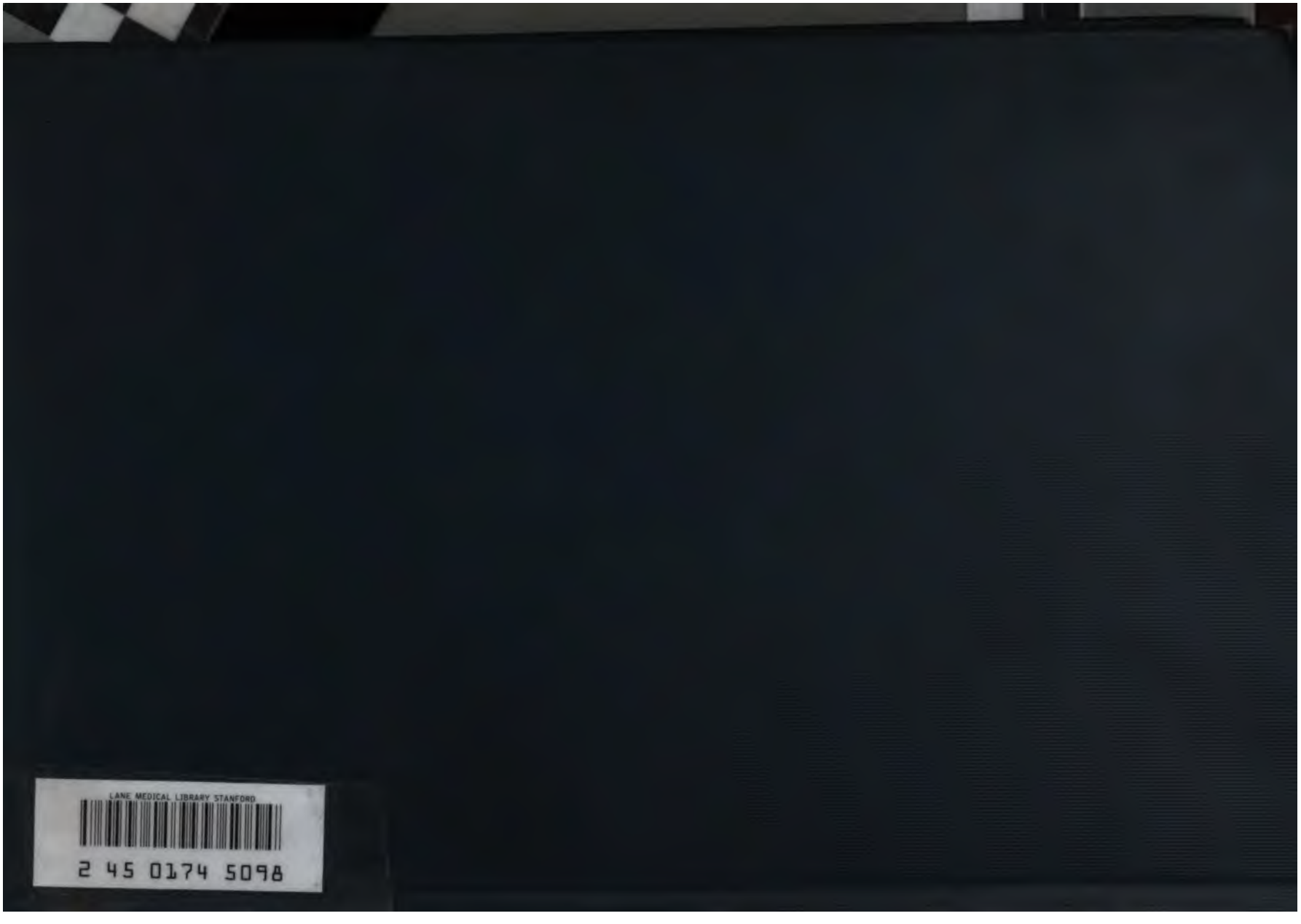
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STUDIES IN IMMUNIZATION AGAINST TUBERCULOSIS

STUDIES IN IMMUNIZATION AGAINST TUBERCULOSIS

BY

KARL VON RUCK, M. D.

AND

SILVIO VON RUCK, M. D.

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"The ancient Jews used to say, that a man does not fulfil his duties in life, who passes through it, without building a house, planting a tree, and leaving a child behind him. A physician, in like manner, should consider his obligations to his profession and society undischarged, who has not attempted to lessen the number of incurable diseases."

BENJAMIN RUSH in *Medical Inquiries and Observations.*

ERRATA

Table facing page 187, second set of entries for children 2-16 years, under column headed "Average Lbs.," first entry should read "3.6" instead of "8.6."

Page 280, lines 6 and 5 from bottom should read "exercise in the open air, to all of which the vegetative processes of the living organism react in a favorable manner."

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PREFACE

It has long been our intention to write a short treatise on the specific treatment of tuberculosis, which should deal with the practical application of tubercle bacillus-products more fully than was possible in our contributions to medical literature and in our periodical reports from the Winyah Sanatorium. Such a book, which should present the collective results of our experience extending over many years, has often been asked for by our friends; but in the past we could respond to many insistent requests only with promises, the fulfilment of which were conditional on time and opportunity.

In preparing a second report from our Research Laboratory, on our studies in prophylactic vaccination against tuberculosis, it became necessary to include the therapeutic results which have been obtained with our vaccine, in infected and tuberculous children, since the first report was published in 1912. When the manuscript of this second report was completed, the opportunity for carrying out our intentions, and complying with our promises, appeared to be favorable, since this report could serve, with some additions, as a practical basis for the consideration of the entire subject.

A chapter on immunity and on the application of its principles for the elucidation of practical questions in tuberculosis thus became necessary. We have, however, entered into the subject only so far as we deemed it essential to enable general practitioners to follow us, who have not pursued special studies in immunity. We have endeavored to discuss the subject in as simple a manner as possible, and have purposely refrained from entering into the more intricate biological problems which concern the laboratory worker rather than the practising physician; for the same reason we have not hesitated to make frequent repetitions wherever they served to explain the subjects under discussion more fully. Those who are well versed in immunology and examine our treatise critically, should bear in mind our object, which was to aid the general practitioner to understand and apply, in his own prac-

tise, the principles which govern the artificial production of bacteriolytic immunity in tuberculosis.

We were guided by similar considerations in our treatment of many practical questions. In discussing the selection of cases which are suitable for specific treatment, the limitations of benefits to be expected from it, the analysis of symptoms, etc., we had in mind the general practitioner who lacks the large experience and wide opportunities of those who limit their practise to the treatment of tuberculous disease.

The subject of tuberculin was discussed before the American Medical Association for the first time at its Washington meeting in 1891. Diametrically opposed clinical results were then reported and admitted by those who took part in the discussion; and the failures and dangers, which had been noted, were urged against the further employment of this remedy. At that time, one of us closed his remarks with words of caution against forming hasty conclusions, insisting that, inasmuch as unquestionable benefits had been shown as well as failures, the proper course would be to continue the study of the remedy, with a view of selecting cases, in the future, in which all conditions appeared to be equal to those in which the favorable results had been observed, as far as it was possible to determine them beforehand.

This manifestly logical attitude has governed us ever since in our clinical use of specific products of the tubercle bacillus. Our observations have fully justified it and have encouraged us to persist in our studies and investigations.

The pessimism which had developed then, and which became more intense with succeeding years, for a long time left us practically alone in our open advocacy of the specific treatment of tuberculosis. The clinical results, which we could urge in its favor, were either ignored or they were attributed to the dietetic and hygienic measures and to the institutional care which were necessarily associated in the treatment of our patients. The inadequacy of these general measures in the treatment of disease is, however, generally recognized and was recently emphasized by Professor Kocher, when he declared that *"it would not be a good sign for the medical teacher and investigator, if he knew nothing better for the treatment or for the cure of disease, which after all form the essence of our*

calling, than 'air, water, rest, and good food'—things which are useful for everybody.'' It goes without saying that we acknowledge the necessity of these general methods as self-evident; but we hold that the far-reaching therapeutic nihilism, which still prevails and which has let it become almost a dogma that tuberculosis must be treated with "air, water, rest and good food" and that active remedial measures are of comparatively slight value or negligible, is to be deprecated if it excludes the administration of drugs or of specific remedies that may be of benefit to the patients. The possibility of influencing the tuberculous, and also the associated pathological processes favorably by suitable specific remedies, has been established firmly, and it may be condemned almost as much, to withhold specific treatment from a tuberculous patient who might benefit from it, as it is to treat a case of diphtheria without antitoxin.

The general measures have been employed in a world-wide crusade against tuberculosis with a view to its delimitation and prevention, although at best they can have only a general effect and cannot secure any specific protection against tuberculous disease. These general measures are applied both in treatment and in prophylaxis on the theoretical assumption that their enforcement will strengthen the organism against the pathogenic action of the tubercle bacillus. Yet, in dealing with the tuberculosis problem in all its phases, it is not permissible to have recourse solely to theory. We may agree theoretically with Sir James Grant, that "wash and be clean is actually the sum and substance of sanitary science"; but we submit that medical science has more specific tasks to fulfil, and that the concrete case of disease cannot be treated exclusively by putting into practise the theorems of sanitary science, but that it demands concrete measures. For the same reason we affirm that the theoretical basis for the general dietetic, hygienic and climatic treatment and prevention of tuberculous disease is not sufficient to justify us in limiting our therapeutic and prophylactic efforts to these general measures, and we claim that every other means, which enables us to hasten the recovery of the individual patient and to protect the exposed individual against the consequences of infection, is justified and proper.

The efforts of the Tuberculosis Crusade are intended (1) to

protect normal persons, especially children, against exposure to infection, and also against its consequences; (2) to arrest and cure early cases of tuberculous disease, before they become open and constitute sources of danger for others.

In urging specific prophylactic immunization against tuberculosis, Krämer compares the past and present efforts of the anti-tuberculosis crusade to fighting the smoke instead of the fire, and other authors have expressed even more condemnatory opinions of its methods. In spite of the insistent and sweeping criticisms which have been leveled against it in recent years, we believe that the campaign has been productive of much good, although we recognize fully that its greatest activities were necessarily directed against results rather than causes. We insist, however, that the power for good of this great movement could be much augmented by directing its efforts deliberately against the cause of tuberculous disease through specific protective immunization. In this event it would not be necessary to invoke the impossible Utopia of a "tubercle bacillus-*free*" generation as a desideratum, for this could fall before the first onslaught of a stray-infection which might develop by adaptation of any acid-fast bacillus to the human and animal organisms. On the contrary, it would tend to render present and coming generations tubercle bacillus-*resistant*, and to protect them against the pathogenic action of the bacillus.

The best way to fight a universal bacterial danger is not by running away from it, even if it were possible; but rather by acquiring the necessary specific protection through which the bacterial infection can be limited and rendered harmless. In this manner the struggle against smallpox was won, and its application to the prophylaxis against typhoid fever and diphtheria has recently been undertaken with full justice. In the same manner only can we hope to be successful in the crusade against tuberculosis.

Under ordinary circumstances the efforts of the tuberculosis crusade cannot bear appreciable fruit for many years; but we are convinced personally that its object could be attained with greater rapidity, certainty and uniformity by a general application of the method of specific prophylactic immunization proposed by us. We can see no reasonable objection to this, especially if the general measures are continued as we nat-

urally would expect them to be. In our own and in the experience of others, the results of immunization with our vaccine, in individuals who were already infected, were remarkably uniform, consisting in general improvement and in the disappearance of signs and symptoms of the disease, which had been present before the method was applied. There being apparently no danger connected with the administration of a suitable vaccine, it is not necessary, for its adoption, to await the concurrence or approval of all interested. This was not done in the case of vaccination against smallpox, against typhoid fever and against other infectious diseases; nor even in the application of passive immunization against diphtheria, all of which methods encountered objections and opposition.

In the pursuit of our researches and experiments, we have never been oblivious of the fact that conditions in the laboratory and in the clinic often differ widely, and we have therefore never advanced conclusions, based upon the results of our experiments, which did not appear to be supported by practical observations of our own, and by those of other experienced clinicians. At all times our aim was to improve the available methods of curing tuberculous disease, and fully as much to find a means for its prevention, even though we realized that these means might not be proved and vindicated immediately.

In referring to our own work, in the specific treatment and, more particularly, in the specific prophylactic vaccination against tuberculosis, we find it necessary to correct a mistaken impression which seems to have been fostered widely, namely, that our method rests upon a purely theoretical basis and that it was developed entirely on the ground of animal experimentation. This is an error. The impetus for our experimental work was supplied by long-continued clinical observations, and its results were invariably controlled and verified by clinical tests. Our method was published only after it had been proved effective and free from danger in a large number of individuals.

The fact that certain authors have not succeeded in similar experiments, does not prove ours to be at fault. Like in other endeavors, success depends upon special experience and skill, and it is indispensable that the conditions under which parallel

experiments are made must be equal. In testing the resistance to an infection, it is, for instance, not permissible to employ the virus in a tenfold infectious dose as was done by one experimenter, or to make the experiment upon diseased animals. The truism that a positive result by far outweighs numerous negative results in importance, applies with particular force in the interpretation of observations of those who lack in special experience or who conduct their investigations under the influence of pronounced skepticism or of personal bias.

Inasmuch as it is the results from the practical employment of a method that must ultimately constitute the deciding factor in establishing its value in the human subject, regardless of the outcome of experiments upon animals, it is within the reach of every clinician to form his own conclusions in regard to the one proposed by us, since the administration of the vaccine has been found safe in our own hands and in those of many others. As it has been found equally reliable with tuberculin for diagnostic purposes, the importance and value of which are generally acknowledged, the clinician can readily determine the correctness or otherwise of our observations that, when this test is made with a suitable vaccine, which contains the body-substances of the specific bacillus in proper proportions and in solution, not only does a reaction occur in the infected and tuberculous organisms, but a complete immunizing response is activated at the same time, which becomes manifest, in suitable cases, by the rapid disappearance of the clinical signs and symptoms of a preceding infection, whereas this immunization is not produced when the diagnostic test is made with tuberculin.

Our experiences in this direction can be verified to particular advantage by applying the test in children in institutions, in which it is desirable to find the tuberculous or the infected inmates, for the purpose of eliminating possible sources of infection, and also of restoring subnormal children to health. Such institutional studies are of the greater value, because the vaccinated children can be kept under continued and prolonged observation. While it is true that clinical investigation requires more time and more extended study than animal experimentation, it must be admitted that the prospect of establishing an effective method of prophylactic vaccination against

tuberculosis justifies any trouble and sacrifice that may be called for.

In citing literary references in connection with the various problems which we discuss in our book, we have made use only of those writings which have a direct bearing upon our work. As this work had a specific and sharply defined aim, and we desired to submit its results to the medical profession, it could not serve any particular purpose to enter into the literature more extensively than we have done.

We do not wish to send out this volume on its mission of service to the practising physician, without making proper acknowledgment to all our coworkers in the Winyah Sanatorium, in the Laboratory and in the Library, whose devotion to our researches, and whose unfailing enthusiasm in the face of many difficulties and discouragements, have aided us in collecting and applying our experiences, in drawing useful inferences and in preparing the present volume.

Asheville, N. C., March, 1916. THE AUTHORS.

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PART I

THEORETICAL CONSIDERATIONS

CHAPTER I

INTRODUCTION. THE TUBERCULOSIS PROBLEM

When with the discovery of the tubercle bacillus as the primary cause of tuberculosis, in 1882, the last link in the chain of evidence had been forged, which had received such signal contribution and support by the investigations of Villemin (1865) and which definitely proved the infectious nature of tuberculosis, the problem that yet remained to be solved appeared to be simple. Since tuberculosis was shown to be an infectious disease, caused by a definite microörganism, the bacillus of tuberculosis, which could be transmitted from person to person, it seemed only necessary to destroy this bacillus and to eradicate it in order to suppress tuberculous disease. But this same task, the accomplishment of which was undertaken by investigators in all civilized countries, soon proved to be far more formidable than had been anticipated and, in fact, impossible of accomplishment. With the growing knowledge concerning the mechanism and effect of tuberculous infection, and concerning the establishment of tuberculous disease, especially phthisis, as well as the development of tuberculosis-immunity, it was found that infection with the tubercle bacillus constitutes only one factor in the etiology of tuberculosis and that many other agencies contribute in bringing it about. As some of these factors are intimately connected with environment in the widest meaning of the term, the efforts directed against the tubercle bacillus and against tuberculosis had to become more comprehensive and more generally educational; they needed to include the betterment of social economic conditions of the poor and of the laboring classes for the purpose of securing a better obedience to the laws of general and personal hygiene; they also had to be extended so as to secure the cooperation of the tuberculous and of their families in attempting the destruction and disinfection of tuberculous material, and in otherwise limiting the various sources of

mission by inaugurating suitable methods of treatment at a period before tuberculous discharges and other tuberculous materials could become sources of danger. The problems of prophylaxis were taken up not only by physicians, but by public-spirited men and women, by municipalities, by national governments, until the movement assumed an international scope.

While medical men in all countries were occupied with the scientific study of tuberculosis, with its etiology, diagnosis, treatment and prophylaxis, the practical application of the results of these investigations met with many difficulties which had to be overcome. At the first French Tuberculosis Congress, which convened in Paris in 1888, and in which for the first time in history the entire deliberations of a great medical congress were devoted to the study of a single disease, it was decided to publish leaflets containing instructions in popular language concerning the prevention of tuberculous disease. At the next congress, Paris, 1891, Professor Armaingaud, of Bordeaux, offered definite propositions in regard to these instructions, and through his initiative the French LIGUE CONTRE LA TUBERCULOSE was founded, upon the first year's activities of which he made a report to the third Tuberculosis Congress (Paris, 1893). By means of pamphlets which were distributed broadcast, and of conferences all over France, the public was being instructed in the elementary principles of hygiene, sanitation, and prophylaxis. Armaingaud's efforts served as a model for most other nations, as they instituted similar crusades of popular education.

The scientific side of tuberculosis-studies had received attention in special publications, especially the "*Etudes expérimentales et cliniques sur la Tuberculose*," of which three volumes were published, between 1887 and 1892, under the direction of Professor Verneuil; also in the transactions of the four French tuberculosis congresses (1888; 1891; 1893; 1898), and of course in the medical literature of all countries.

In the meantime the sanatorium idea, initiated more particularly by Pidlington in England and by Herrmann Brehmer in Germany, had overcome the objections which had at first been raised against it and under which both these pioneers had suffered severely, and it had obtained a firm foothold. Detweiler,

a former assistant of Brehmer, who had recovered from tuberculosis in the latter's institution in Görbersdorf, had founded the sanatorium Falkenstein (1876) in the climatically ideal Taunus, and also the first sanatorium for the poor, in Rupperts-hain (1891). As early as May, 1884, a committee of the *Doktoren Collegium* in Vienna had, at the instance of Professor v. Schrötter, memorialized the Austrian Government and the city authorities of Vienna, asking them to establish and maintain, in the neighborhood of the city, a large tuberculosis-sanatorium in which the very considerable tuberculous portion of the hospitalized population of that city (about 3,300 per annum) might receive the benefit of open-air treatment and might incidentally supply the clinical material for scientific studies and researches[1]. Although the city of Vienna did not then act upon this suggestion on account of the very considerable expense involved in the project, an association, VEREIN HEILANSTALT ALLAND, was founded privately, which had for its purpose the founding and maintenance of a climatic station for tuberculous patients. This association received official sanction on August 10, 1890, but did not succeed in carrying out its program for a number of years.

Following the foundation of the French League against Tuberculosis through Professor Armaingaud in 1891, similar associations were founded in the United States, in Belgium, in Germany and elsewhere, which had the purpose of popularizing the practical lessons to be learned from scientific research, to teach people that tuberculosis is communicated by direct infection and is therefore preventable, and that its progress and ill-effects can be alleviated, if not entirely prevented, by proper living and especially by open air-treatment. The idea which Professor v. Schrötter had attempted to realize in 1884, viz., that the consumptive poor should be cared for in sanatoria and that in these institutions the disease, but more particularly its prophylaxis, should be studied, received a new impetus when he made the proposition to the fourth French Tuberculosis Congress, that it should appoint an international and permanent committee for the study, and principally for the prevention of tuberculosis.

In Germany, a Central Committee had been organized in

[1] v. SCHRÖTTER. C. R. Congrès pour l'Etude de la Tuberculose. 4e session. —1898. Paris, 1898; p. 193.

1895 for the purpose of erecting and maintaining sanatoria for consumptives, in which they might derive the benefits from dietetic and climatic (open-air) treatment which Brehmer in Görbersdorf and Detweiler in Falkenstein had shown to be possible. This Central Committee was to guide and to centralize the work of the many local leagues which had been formed throughout the German Empire for the benefit of the consumptive poor and also of the working classes. The work of these leagues and of the Central Committee was popular rather than scientific, and one in which the results of scientific research were to be put in practise. The Committee therefore decided to convene a congress in Berlin, in the spring of 1899, which was to consider the problems of tuberculosis as a disease of the masses, its nature, its dangers, and the means for the struggle against it, especially its prevention.

The Berlin Tuberculosis Congress took place in May, 1899. It was followed by a similar congress held in Naples, in April, 1900, and by the British Congress on Tuberculosis, for the prevention of consumption, held in London in July, 1901.

At the time when the Berlin Tuberculosis Congress was held, sixty public sanatoria were already in operation throughout the German Empire, the work of which was greatly assisted and facilitated by the far-reaching system of compulsory insurance of working people against sickness and incapacity, which is in force in that country. By means of this system, the working man, when attacked by tuberculosis, has a legal claim on insurance-funds, for medical treatment and maintenance in his own case, and for the support of his family. Thus it comes about that he can, without forfeiting in any degree his sense of self-respect, procure at once, at the very onset of the malady, treatment requisite for himself and at the same time maintenance of those depending upon him.[2]

In England, the hospitalization of consumptives and the prophylaxis of the disease were of older origin and had already commenced to show results. Between the year 1791, when the Royal Sea-Bathing Infirmary for Scrofula was erected at Margate, and the year 1886 when the Mildmay Convalescent

[2] cf. H. Timbrell Bulstrode. Report on Sanatoria for Consumption and certain other aspects of the Tuberculosis Question. London, 1908; p. x.

Home in Torquay was founded, seventeen other hospitals for consumptives had been established and operated and, in his memorable London address in 1901, Professor Koch attributed the remarkable fall in the tuberculosis death-rate, which had taken place in England, to these institutions which have multiplied greatly in the last twenty years. Undoubtedly there have been other factors of equal importance. It is interesting to note that the "sanatorium idea," i. e., the treatment of consumptives in closed institutions in which the greatest possible degree of open-air life and of proper, i. e., plain and nutritious diet, as well as constant medical supervision is maintained, took its inception in the Highlands of Scotland as far back as 1747 and was developed among others by such men as Bodington (1840) and MacCormac (1855), the latter of whom was "intimately and entirely convinced that the disastrous and wretched malady . . . is not only when taken early, very often removable, but, what is of still greater importance, that with proper means and appliances it is in every single instance preventable."

In the United States the educational movement, which has been notably effective, commenced with the foundation of the Pennsylvania Society for the Prevention of Tuberculosis, in 1892, and made rapid headway; it is organized in the voluntary associations for the prevention of the disease, which have been formed during the last twenty years in all parts of the country. When the NATIONAL ASSOCIATION FOR THE STUDY AND PREVENTION OF TUBERCULOSIS was founded, which held its first annual meeting in Washington, in May, 1905, various state boards of health, municipal departments of health, private institutions and local societies had been active in the crusade against the disease for several years.

The meeting of the Sixth International Tuberculosis Congress, in Washington, D. C., in 1908, afforded a favorable opportunity for a survey of existing conditions, and it was found that 195 associations were in existence in the United States. According to the last Tuberculosis Directory, compiled for and published by the NATIONAL ASSOCIATION, in 1911, there were at this time 500 such societies representing all parts of the country and charged with the responsibility of dealing with the problem in their respective states and communities. This directory records an increase of 178 special institutions for the care of tubercu-

lous persons, over the number listed in 1908, with an increase in capacity of from 14,000 to 26,360 beds.

In addition to 453 tuberculosis sanatoria and hospitals, and 338 tuberculosis dispensaries, as well as day camps and night camps for adults, the directory lists 69 hospitals for the insane and 29 penal institutions which make special provisions for the care of tuberculous inmates. There were also 52 open-air schools and classes for children being conducted in 1911, which have since then been increased materially.

All tuberculosis societies in the United States are centered in the NATIONAL ASSOCIATION which is in touch with the greater crusade through the INTERNATIONAL CENTRAL BUREAU at Berlin and has, in its annual meetings, given evidence of the great and splendid work being done in our country.

The crusade against tuberculosis, in the United States, owes much to the initiative and enthusiasm of Dr. Edward L. Trudeau. The ideas which Brehmer had preached in Germany for almost twenty years, namely, that certain cases of pulmonary tuberculosis do well in open-air life, with good and wholesome food and with much rest, had worked well in his own case, and he founded, in 1884, the Adirondack Cottage Sanatorium for the Treatment of Incipient Tuberculosis in Working Men and Women, as a semi-charitable institution, for the purpose of applying the same idea in other cases.

This sanatorium was the first institution of its kind to be established in the United States, although "Homes" for consumptives had been maintained for years in several cities, more particularly for the benefit of incurable, indigent consumptives. It is of interest to mention in this connection that the belief in the benefits to be derived from an open-air life dates back, in our country, to Benjamin Rush, whose "Thoughts upon the Cause and Cure of Pulmonary Consumption"[3] center in this idea. In his opinion the open-air life was to be combined with a carefully regulated, increasing regimen of exercise.

The great prevalence of tuberculous disease in its open stage and, consequently, the great opportunity for exposure to infection, led to the idea of the so-called *ubiquity* of the tubercle bacillus, according to which the bacillus is generally prevalent

[3] BENJAMIN RUSH. Medical Inquiries and Observations. Philadelphia, 2nd edition, 1805; I., p. 201.

in nature, and contact with it cannot be avoided. The error of this conception has long been demonstrated; we know that the tubercle bacillus cannot propagate outside of the living body and that one can speak of "ubiquity" only in the sense that the majority of people come in contact with it some time during their lives. The term is still being employed, however, by physicians and medical writers without qualification, which has the tendency to perpetuate the error existing in the popular mind. The manner in which the constant and ever-present danger of infection was insisted upon very naturally caused the public to believe that this danger refers not only to public and private rooms and other closed places occupied or frequented by phthisical persons who expectorate tubercle bacilli. but it was inferred that it exists also in the open air. The findings of Cornet, who first demonstrated virulent tubercle bacilli in the dust and sweepings in rooms occupied by consumptives, and those of Flügge, who showed that tubercle bacilli can be discharged on speaking, coughing, sneezing, and can float in the air for a short time, were generalized and taken to prove that the atmosphere is liable to be contaminated with tubercle bacilli and that this contamination is increased in the proximity of the abodes of tuberculous subjects. A person in any stage of tuberculosis is presumed to endanger others whom he passes on the street and who live in adjoining houses or even across the street, the bacilli being conveyed, supposedly, in the air within a certain distance and being apt to infect the passer-by, or to invade neighboring dwellings. So firmly has this prejudice become established that law courts have attempted to regulate the distance from other buildings or dwellings within which a hospital, sanatorium or dispensary for tuberculous patients may be established, and municipalities have attempted to pass similar regulations in the supposed interest of sanitary policy.

It is our opinion that many of those members of the medical profession, who have contributed in engendering this state of fear and alarm, have done so in their zeal for promoting the cause of prevention, and in their endeavor to cause the measures advocated for the care and disposal of tuberculous secretions and excretions to be observed more conscientiously. When the etiological relation of the tubercle bacillus to tu-

berculosis was established without a doubt, the fact of its communicability was preached and insisted upon without regard to the actual mechanism and modes of infection, and especially in the anti-tuberculosis crusade for popular education, the public was led to believe that the contagiousness of tuberculosis was identical with that of smallpox, diphtheria, scarlet fever or influenza. It is but natural that supposedly well persons came to shun consumptives and to avoid coming in contact with them; it is also natural that they should fear the consequences of living in close proximity to the dwellings of consumptives, or to institutions for their care and treatment, inferring that such a proximity would lead to the infection of the entire neighborhood. The fear of infection ran riot and went astray to such a degree, not only among the laity but also among physicians, that such peculiar assertions could be made in all seriousness as the one that tubercle bacilli were conveyed from the bodies of persons dead of consumption, after burial, and that they contaminated the sod and grass of cemeteries; that they were therefore likely to reach the air and to cause infection.

Having led the public astray by overdrawn teachings and statements, the medical profession must now assume the duty to correct mistaken ideas and views, to undo the effects of imperfect and one-sided instruction by drawing logical inferences from the established facts, for the proper guidance of the public mind. Above all it is to be insisted upon that ALL ATTEMPTS TO DEMONSTRATE THE PRESENCE OF TUBERCLE BACILLI IN THE AIR OUTSIDE OF BUILDINGS OCCUPIED BY PERSONS WHOSE DISCHARGES CONTAIN THEM, HAVE FAILED INVARIABLY, there being not a single instance on record in which a genuine tubercle bacillus has been found in the open air.

The fear of the omnipresent danger of infection borders on the ludicrous when we consider that tuberculosis-infection occurs invariably indoors, in closed rooms and places occupied or frequented by consumptives, and that as a rule it occurs in early life. Even in such cases the questions of frequency and duration of exposure, the number of living tubercle bacilli which are inhaled or ingested, and the degree of their virulence, are of deciding importance for the actual occurrence of an infection which leads to disease; further, the fact is to be taken into consideration that, of all persons who are infected, only a

minority develop clinical tuberculosis, which goes to show that the human species possesses a fair degree of resistance to the pathogenic action of the virus of tuberculosis.

As for the fear of sanatoria and hospitals in which tuberculous persons are cared for, experience has shown that, when properly conducted, these institutions are in fact safer to live in, as regards the danger of acquiring an infection, than are other public places. The patients living in tuberculosis hospitals and institutions are instructed in, and obliged to observe the precautions which are required to prevent their endangering others; they are taught how to take care of the infectious materials which they discharge and how to make them innocuous; the disinfection and sterilization of rooms, linens and utensils, etc., is in experienced hands and is carried out conscientiously. It has been established years ago and is being verified constantly that physicians, nurses, and also the general employees in tuberculosis hospitals and sanatoria do not develop tuberculous disease more frequently than is observed generally, and that, if a difference of frequency exists, it is in fact in their favor. This is recognized by life insurance companies, since they do not usually consider the prospective risks to be greater for members of the resident staff and the employees of a sanatorium for tuberculous persons than for persons who are employed otherwise or live elsewhere, or in their own homes. While a very few of the companies hold such residence to be prejudicial, the majority do not; one of the most conservative companies in this country even decided that, other things being equal, the risk of medical officers in tuberculosis sanatoria is better than that of the average person.

If the preventability of tuberculosis was insisted upon most strongly in the crusade for popular education, and if it had, among many excellent results, the undesirable effect of developing the phthisiophobia which we have just discussed, and of causing consumptives to be regarded almost as criminals who are to be shunned, the work of the tuberculosis crusade has nevertheless been an important factor in arousing the popular mind to the importance of public prophylaxis as an economic asset. The various local, national and international leagues and associations against tuberculosis or "for the study and prevention of tuberculosis" have become an immense power for

good all over the civilized world, and have contributed particularly to the earlier recognition and treatment of the disease. It is being realized more and more that, the earlier in the tuberculous process the diagnosis is made, the better are the chances for a lasting arrestment or even cure. It is further being realized more and more, that the best mode of dealing with the tuberculosis problem is by preventing the disease, and in all walks of life, in shops, factories, stores, assembly halls, etc., means are taken to counteract the evil effects of crowding, while in the industries those occupations which notoriously predispose to the acquirement of tuberculosis are provided with proper appliances for the suitable elimination of the dangerous factors, especially the inhalation of dust.

In these efforts of prevention, the importance of childhood prophylaxis is now recognized with particular emphasis, since the researches of the last fifteen years have demonstrated the fact that the seeds of adult-phthisis are usually laid in childhood, and that the infection with the tubercle bacillus occurs, in the majority of cases, during the early years of life. The necessity of protecting the children against the acquirement of tuberculous disease is therefore very great and is receiving increasing attention.

French authors insist particularly upon the importance of the receptive soil for infection, and upon the unfavorable influence exercised on this soil by over-exertion, environment (crowding in unhygienic conditions), and alcoholism, an unholy trinity to which may be added insufficient nutrition. It was to these four "principal causes of tuberculosis" that Professor Hérard called the primary attention of the Paris Congress for Tuberculosis in 1905 in his presidential address.[4]

In its practical and general phases, the world-wide warfare against tuberculosis might be said to have been instituted with the Berlin Congress in 1899, although it had undoubtedly received its impetus through the preceding four French congresses. It is unique and without a precedent in history, there having never before been such concerted and continued action to oppose the ravages of any one disease. It has since found its counterpart in the crusade against venereal diseases and also

[4] HÉRARD. Congrès international de la Tuberculose, tenu à Paris, du 2 au 7 Octobre 1905. Paris, 1906; tome I, p. 31.

in that against cancer, the latter of which, however, is as yet only in the stage of scientific research, since we have no knowledge that might guide us in the prevention of cancer.

Unfortunately the results of the tuberculosis crusade cannot become undeniably manifest for a long time to come, because it is directed against effects rather than causes, and voices have been raised repeatedly against its mode of action. So Cornet and others denied a few years ago that the sanatorium movement had accomplished any material results in Germany, and more recently the justice and value of the present methods of fighting tuberculosis in this country have been questioned insistently.

One of the authors, who deny the effectiveness of the present methods, collected a mass of statistics bearing on the mortality from consumption and including a number of cities, in the United States, with a total population of about 16,000,000. In comparing the mortality statistics from consumption, he says, the fact must, of course, be recognized that, as a rule, the disease has been diminishing gradually in probably every city from its earliest records to the present time, and this long before the current preventive measures had been instituted, chiefly because of improved general sanitary conditions brought about by wise legislation. Little, if anything, was done before 1894 in regard to preventive measures of the present era, and, on comparing the first half of the total records of thirteen cities, covering a period of thirty-five years, with the year 1893 as the central year, this first period (1875-1893) is taken to demonstrate the rate of diminution in the ante-prevention period, that is, what the author calls the "normal rate of decrease," while the second half (1894-1912) covers the period during which the preventive measures were in active operation. In these thirteen cities the so-called normal rate of decrease, from 1875-1893, was found to be higher than that from 1894-1912, suggesting that the so-called normal decrease in the death-rate from consumption was more favorable than that assisted by modern prophylactic measures.

According to the author's conclusion, the trend of the evidence, which he presents, is strong enough to force the conviction that the decrease of the death-rate from consumption has not been accelerated one whit by the preventive measures

which have been evoked during the last ten or fifteen years; on the other hand, he continues, there are positive indications that, on the whole, the number of deaths from this disease have, for some unaccountable reason, increased during the years when these measures were in full sway, involving immense efforts and the expenditure of money amounting in a single year to over twenty million dollars in the United States alone. He claims that the only remedy, which has so far been found effective in reducing the number of deaths from this disease, consists in the improvement of our physical and mental environment, and in the moral betterment of the human race.

It will be of interest to study similar statistics, at least for the period of the so-called normal diminution-rate, of the tuberculosis mortality in England. Before the Epidemiological Society in London, a paper was read, in 1898, on the prospect of abolishing tuberculosis, in which the author stated that the annual death-rate from phthisis in England, had declined steadily from 3.8 per thousand living in 1838, to 1.3 per thousand living in 1896. The first great fall was observed in the period of 1840-1850, when land-drainage was being carried out extensively. The mortality then remained nearly stationary until 1867, since which time the decline has been continuous, corresponding with the sanitary improvements, the better ventilation of houses and workshops, and the higher standard of living among the working classes. For the future, this author considered the outlook encouraging, and he believed that judging from the success that had already attended past efforts for the general improvement of public health, and from the great though undesigned reduction in the mortality from tuberculosis, which had followed, "*England might confidently look forward to the practical extinction of this disease at no distant future,*" as the result of the persistent cooperation of the sanitary authorities, the medical profession, and the public, in measures intelligently directed to the end in view.

It is not necessary to more than direct attention to the contrast between the first pessimistic, and the second optimistic subjective views of these two authors. The former's idea, that the efforts of the last twenty or thirty years had produced no results, is just as ill-conceived and mistaken as is the sanguine confidence of the second author, who forecasts the eradication

of tuberculosis at no distant future. It will be seen that the most decided diminution in the tuberculosis mortality in England is attributed to definite causes, namely, the extensive land-drainage of 1840-1850, and the general improvement in sanitation and in the standard of living in 1867-1898. Between 1850 and 1867, when no particular factors were active, the rate of mortality from phthisis remained practically stationary. It is therefore quite probable that the relatively greater diminution in the tuberculosis mortality of the American cities, before 1893, may also be referred to definite causes, and that the less favorable results since 1894 occurred in spite of modern preventive measures, which after all are simply those improvements in sanitation and hygiene and in the standard of living, which are dictated by common-sense, plus special precautionary measures resulting from our increasing knowledge of the origin and the mode of distribution of the disease.

Even then the seeming failure of modern preventive measures, to produce a greater diminution in the tuberculosis mortality, may be taken as emphasizing the vastness and immensity of the problems involved. On the other hand, there is no doubt that, by a like statistical method, figures could be adduced, if it were worth the while, which would give more encouragement for the past efficiency of the crusade, to those who have given their time, efforts, and financial support in this work for the benefit of mankind, and which would encourage the continuance and the increase of these efforts, and the enlistment of others in the cause.

The vastness of the task confronting us in undertaking to stamp out, or even to materially reduce, the prevalence of an infectious disease after it has spread practically all over the world, over which it held sway as long as we have history, must be apparent to every one, and under the circumstances, after little over two decades of concerted effort, the conservative student of the subject would hardly be justified in expecting so evident a reduction in mortality or even in morbidity, that the statistical proofs were incontrovertible. Such a result would be the more surprising at the present time, when one bears in mind that, unlike acute infections and epidemic diseases, a tuberculosis-infection need not become manifest for many years after its occurrence. If, as we believe with others, tuberculosis-in-

fection takes place during early childhood in the great majority of cases, it is reasonable to take the position that, in the present available statistics of deaths from tuberculosis, the infection usually antedated the inauguration of the more general adoption of restrictive efforts, and that it is premature to expect any manifest results which could be credited to these efforts.

We believe therefore that, at the present and for some time to come, mortality statistics cannot be cited either for or against the success of preventive efforts against actual infection and disease, but that they reflect chiefly the results of hygienic and general efforts by and through which the already existing infections may be prevented from reaching a stage of manifest disease. This is also true for the results of the special activities of public and private sanatoria, dispensaries and other provisions for the treatment of actual cases of tuberculous disease. Germany having the greatest facilities in this respect, the preventive influence may be expected to manifest itself more particularly in that country and, in fact, statistics of mortality are frequently compiled by German writers in support of the view that the lowered tuberculosis mortality, which actually obtains, stands in relation to the special prophylactic efforts directed against the disease.

Unfortunately a large number of patients, treated by the general methods in vogue, relapse in the course of time, and their deaths, being only delayed, will of course continue to keep up the mortality-rate, although it must be conceded that the arrestment of early cases lessens, for the time, the number of persons that may be subjected to infection through their agency. On first consideration the claim is a reasonable one that the work done by sanatoria and dispensaries, and the influence of the educational propaganda by these institutions and by the general enlightenment of the public, in regard to sputum-prophylaxis and to other prophylactic measures calculated to limit and to prevent infection, must have an influence which is of great importance and is far-reaching. It is not subject to statistical demonstration what this influence may have been up to the present time, and what it may be now over thirty years after the modes of conveying tuberculosis-infection have been recognized and understood. In the absence, and because of

the impossibility of the most effective measure of prevention, which is ABSOLUTE ISOLATION of infected individuals or at least of those who can convey the germs of the disease to others, we may, however, well look into what evidence may be available, in considering to what extent, if at all, our general warfare against tuberculosis has lessened or is likely in the future to diminish the FREQUENCY OF INFECTIONS and the frequency of the clinical disease. In our study of this, the most important phase of the question, we confess to being apprehensive that, even under favorable sanitary and hygienic conditions, tuberculosis will prove no exception to other infectious diseases, in all of which, with the sole exception of smallpox because of the specific prophylactic immunization against this disease, isolation of the infected has been the only efficient method to prevent their spread.

While we have no definite proof that, in man, a tuberculosis-infection from outside sources must be repeated again and again in order to produce disease when the conditions for its acquirements are otherwise present, we know that infection with the tubercle bacillus occurs, as a rule, through exposure in house and family, and at all events that it occurs indoors, in consequence of direct or indirect contact with persons whose morbid discharges contain the Bacillus of Koch.

It is also known that the tubercle bacillus is very resistant to destructive influences and able to retain its viability for long periods of time. It is extremely minute, and a single droplet of expectoration may contain it in great numbers, many times that which is sufficient to cause infection. It has been established by the studies of Cornet, Flügge, and others, that living virulent tubercle bacilli may be found in the dust of rooms occupied by persons suffering especially from pulmonary tuberculosis, who discharge them not only with large masses of sputum coming from lung cavities in advanced pulmonary phthisis, but who may eliminate them with their expectoration at a time when their disease has not yet made a marked advance in tissue destruction, when the amount of expectoration is small and when the symptoms of ill-health are too slight or indefinite even to suggest a sputum examination, because there is nothing to connect the existing symptoms with the lungs as the seat of serious disease. Flügge and his coworkers have shown con-

clusively that persons with open pulmonary tuberculosis may project particles or droplets of moisture for a distance of several feet, in speaking, singing, hawking and coughing, in which tubercle bacilli may be found. Although this does not occur uniformly in all cases and at all times at which such experiments are made, we must reckon with all possible means for the direct and indirect conveyance of the infection; direct by inhalation while the droplets are suspended in the air, and indirect after they have gravitated to the floor and after the moisture has evaporated, when the tubercle bacilli are mixed with the floatable dust of the apartments, as Cornet has shown to be possible in his examinations and experiments with dust taken from various places in rooms occupied by tuberculous patients.

These facts having been well established, we should take into consideration what measures have been or can be employed in order to prevent absolutely the presence of free tubercle bacilli in the air of the room or rooms of a person who discharges them in greater or lesser numbers. If it is remembered that few persons are ever removed to public or private sanatoria before a positive sputum-examination clinches the diagnosis of tuberculous disease, when in this manner the presence of tubercle bacilli has been established, it is only just to conclude that the specimen actually examined, in all probability, was not the first one in which a painstaking search would have revealed them. Granting, what is improbable, that a sputum-cup is always used and that its contents are carefully destroyed, it must be admitted that even careful and clean patients can still liberate tubercle bacilli in speaking, hawking, coughing, and that they probably wipe their lips with their handkerchiefs, after expectoration, even though they do not expectorate into them; that in handling their handkerchiefs and sputum-cups they are likely to contaminate their hands and their clothing, and that for all these and other reasons our present mode of sputum-prophylaxis is by no means what must be demanded in order to assure absolute protection against the presence of tubercle bacilli in the air and dust of rooms in houses in which a person with open tuberculous lesions mingles with those who occupy them with him. The precautions as they are now practised are therefore manifestly inadequate, even though they are car-

ried out conscientiously by the more intelligent classes of people; and they must be far more inadequate under conditions of crowding and of a greater want of appreciation of what is required to render a room sterile as far as the tubercle bacillus is concerned, in spite of its being occupied by a person who can and does discharge millions and even billions of these microorganisms in a single day. If, therefore, infection were invariably followed by disease, the outlook for a solution of the tuberculosis problem would not be encouraging.

Realizing that opportunities for infection must occur even under such care as it has been possible to secure in the disposal of tuberculous sputum, B. Fränkel [5] proposed, some years past, the wearing of a mask by the consumptive patient. Inasmuch as this mask would have to be removed when, on coughing, the expectoration is to be discharged into a sputum-cup, and in spite of the fact that it would no doubt lessen the frequency with which tubercle bacilli escape into the atmosphere, the actual protection of those who are exposed to infection cannot be secured by such a makeshift, since it lies in preventing absolutely every escape of tubercle bacilli, and this must be admitted to be an impossibility.

When making reference to the face-mask of B. Fränkel, at the Berlin Tuberculosis Congress, in 1899, Dr. Roth [6] described "coughing-fans" which he had manufactured of hard rubber or of aluminum, against which the patients were supposed to cough and which could be kept clean easily. Eichentopf,[7] a dentist, constructed a face-mask for the protection of those who come in contact with persons suffering from infectious diseases, the germs of which may be disseminated by the exhaled air, and Rönnevig [8] described a mask introduced by E. Grundt, the director of the Sanatorium Lyster, in Norway, which is also recommended for the use of physicians when examining patients.

In hospitals and charitable institutions it may be possible to make the wearing of face-masks obligatory upon the patients,

[5] B. Fränkel; Berl. klin. Woch. 1899, XXXVI, 21.

[6] Roth; Bericht über den Kongress zur Bekämpfung der Tuberkulose als Volkskrankheit. Berlin, 24. bis 27. Mai 1899. Berlin 1899, S. 268.

[7] Eichentopf; Medizin. Woche. 1907, VIII, 193.

[8] Rönnevig; Ztschr. f. Tub. 1912, XIX, 238.

even though their actual advantages in this case are open to question. Persons living in their own homes, and patrons of institutions, in which the patients pay for their maintenance and for medical services, would refuse almost without exception to be "muzzled." In any case, the benefits accruing from such an apparatus would be problematical, and in those cases in which the infectious material is most apt to be scattered through the room or over the bed linen, that is, in far advanced and especially in terminal cases, the patients are usually too ill to wear masks.

As has been stated before, we have known all these things for over thirty years, and, that the public has not been in ignorance of them, is shown by the fear which the consumptive engenders wherever he goes, and which is often manifested in a most unreasonable manner. We have alarmed the public by an alleged, or at least by an exaggerated danger, the supposed extent of which is not warranted by the facts, and we have entirely failed to gain the object sought.

Our own observations and studies, supported by experimental investigations, have convinced us years ago that an actual danger from the lack of efficient sputum-prophylaxis accrues not so much to adults as it does to children, and that the danger is the greater for them, the younger they are. Without supporting this statement by statistical evidences, we do not hesitate to assert, that the protection of a nursling against tuberculosis-infection is rarely possible in a family in which there is a person whose sputum contains tubercle bacilli, and never if this patient is concerned in the care of the infant. Even if the latter is absolutely isolated from all other rooms in the house, and is confined strictly to the nursery which is never entered by the tuberculous person, and if a separate door leads to the garden or street, and even if the infant is attended exclusively by a special healthy nurse, we hold that these precautions may still prove insufficient to prevent infection, in some cases and under certain conditions. We believe that the same holds true, although less rigorously, for children who have reached school age, and from then up to adolescence, whereas in adults the exposures must probably be more severe and the infections more massive or more frequent in order to produce disease. The resistance to the development of clinical disease,

which may be observed to obtain under the most unfavorable conditions and with the most extreme degrees of exposure (of which all experienced physicians could cite instances), may be ascribed to an acquired specific immunity against the bacillus of tuberculosis. To account for such observations, we may assume that a sufficient degree of immunity developed through specific reactions to repeated access to the tissues of the individual of tubercle bacilli which were dead or non-virulent, or the virulence of which was lost or sufficiently attenuated after their discharge from their host. It is also possible that a sufficient degree of immunity may be transmitted from the tuberculous mother to her child, and we have made observations which can hardly have another explanation.

Cornet has shown the frequency of tuberculosis in early infancy to stand in relation to the exposure to infection in the family, while the observations of Epstein,[9] which were made as long ago as 1879, make it clear that no infection occurs without exposure. This author, who was assistant physician to the Foundling Asylum in Prague, found, in 200 autopsies upon nurslings and children mostly under six months of age, that the pulmonary affections usually present were catarrhal pneumonia, bronchitis and atelectasis. In the nine cases of tuberculosis among them, the children had not been received directly from the obstetric clinic, but from their homes at ages of ten weeks and upward. Seven of these nine nurslings were brought to the asylum because the mothers had been removed to a hospital on account of phthisis. One was returned by a foster-mother, herself a consumptive, and in only one case, in which the foster-mother was not examined, was there no evidence of the probable source of infection. In the four years of his service, Epstein has not observed a single death from tuberculosis, in which the infection occurred in the institution.

Bollinger[10] records similar observations from the Munich Orphan Asylum. In a total of 603 children, cared for in the institution, there was not a single infant who became tuberculous, although 50.45 per cent of them had tuberculous mothers. Only one case of tuberculosis was observed in the institution

[9] EPSTEIN; cf. Schmidt's Jahrb. 1879, CLXXXIV, 153.

[10] BOLLINGER, Münch. med. Woch. 1888, XXXV, 479.

and in this case the disease had been recognized as being present on admission.

Professor Hutinel [11] also contributed valuable data in this respect. Of about 18,000 children taken charge of at birth by the *"Assistance Publique"* in Paris and placed with families living in the country, subsequent inquiries were made as to the frequency of tuberculosis among them, because many of them had tuberculous mothers. It was found that only 16 of the 18,000 children had developed the disease.

Comby [12] found that when children of tuberculous parents are removed from their homes to a non-tuberculous environment, only three per cent die of tuberculosis, whereas otherwise their mortality from tuberculosis reaches fifty per cent. This was especially manifest in the well-known clinical experiment related by Bernheim [13] who gives the histories of three pairs of twins, born of consumptive mothers. One of each pair remained in the parental home, being nursed by a healthy woman, while the other twin of each pair was sent to the country and brought up on the bottle. The three children kept at home died of tuberculosis. Those who were removed from their homes remained well.

The influence of the exposure to infection in school is well shown by Hillenberg [14] who found out of 74 children, from 5 to 7 years old, who had just begun to attend school, a positive reaction to the cutaneous tuberculin test in 12.87 per cent, while among 31 children who had gone to school for one year (6-7 years old), 32.57 per cent gave a positive reaction.

The views which we have illustrated are confirmed further by numerous instances in our own experience, in which we had the opportunity of examining critically all or several of the children in the families of patients under our professional care; we can not recall a single instance in which the tuberculosis-infection could not be demonstrated by means of tuberculin- or other biologic tests, in addition to other stigmata in nutrition, enlargement of lymph glands, and not infrequently

[11] HUTINEL; 2e Congrès de la tuberculose; Paris, 1891, p. 344.
[12] COMBY; Arch. d. méd. des Enfants. 1905, p. 651.
[13] S. BERNHEIM; Centrlbl. f. Bakt. 1894, XV, 656.
[14] HILLENBERG; Ztschr. f. Hyg., etc., 1909, LXIV, 310.

by positive physical signs in the lungs, which were found especially in older children.

The insufficiency of present prophylactic methods under the educational campaign of the tuberculosis crusade is made evident further by results of tuberculin tests made within several years past upon large numbers of children.

So v. Pirquet [15] examined 876 nurslings and children up to 14 years of age, in Professor Escherich's clinic in Vienna, and found the following percentages in their responses to the cutaneous tuberculin test:

0–3	months	0	per cent	1–2	years	24	per cent
3–6	"	5	" "	2–4	"	37	" "
6–12	"	16	" "	4–6	"	53	" "
				6–10	"	57	" "
				10–14	"	68	" "

while at the ages above 14 years, the frequency was 90 per cent.

The writer of an editorial, in the British Medical Journal,[16] states that, according to a personal communication from the director of the hospital, 90 per cent of the children, who died of other diseases in the Children's Hospital at Bucharest, were found after death to have been subject to tuberculosis-infection.

E. Ausset [17] found the following response to the conjunctival tuberculin test in 294 children:

Among	13	infants, less than 3 months old			None reacted
"	53	infants up to 1 year old	8	or 15.09 per cent	"
"	126	children 1–6 years old	46	" 36.5 " "	"
"	50	children 6–9 years old	28	" 56 " "	"
"	65	children 9–15 years old	38	" 58.4 " "	"

Hamburger & Monti [18] examined 532 children at the Vienna Children's Hospital, ill with non-tuberculous acute affections, by injecting small doses of undiluted old tuberculin and observing the usual and also the "Stichreaktion." They found no positive reactions in the children under one year of age. After the first year of life, positive reactions were ob-

[15] C. v. PIRQUET; Wien. med. Presse, 1907, XLVIII, 1733.
[16] Brit. Med. Journ. 1907, II, 470, editorial.
[17] E. AUSSET; Revue de Médecine; 1908, XXVIII, 359.
[18] HAMBURGER & MONTI; Münch. med. Woch. 1909, LVI, 449.

[illegible] per cent; [illegible] per cent; [illegible] per cent; [illegible] per cent; [illegible] per cent; [illegible] per cent; [illegible] per cent; [illegible] per cent; [illegible] per cent; [illegible] per cent; 94 per cent,—from which [illegible] that the [illegible] universal [illegible] in the age of 14 years, at least in the hospital population of [illegible], and that tubercu[illegible] [illegible] during child[illegible]. Arranged according to age-periods, the steady increase [illegible] and is found to be as follows:

I. period—	[illegible]	[illegible] per cent
II.	— [illegible]	[illegible]
III.	— [illegible]	[illegible]
IV.	— [illegible]	[illegible]
V.	— [illegible]	[illegible]
VI.	— [illegible]	94

[illegible] tuberculous par[illegible] [illegible] as follows:

[illegible] years positive in	[illegible] per cent
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
14	[illegible]

He believes that [illegible] negative results were [illegible] families in which [illegible] children are [illegible] year of life.

[illegible] found among [illegible] material of 2,000 chil[illegible] who were [illegible] tuberculin test, that [illegible] 78 or 22 per cent [illegible] children of [illegible] gave a posi[illegible] reaction. [illegible] to have [illegible] that is, [illegible]

[illegible] Berl. [illegible]

[illegible] Beitr. z. K[illegible] 1911, XVIII, 117.

Our own observations in over 600 examinations of children, regardless of known exposure, gave more or less evidence of a preceding infection in 70 per cent. In 393 such examinations which were made in children at the Baptist Orphanage at Thomasville, N. C., the relation of family exposure was apparent in this sense that, in instances where two or more children of the same family were among the examined orphans, these children were found to have traces or signs of tuberculosis-infection, or not, accordingly as one or both of the parents had or had not died of tuberculosis.

There is no need to cite further statistics of this kind, as such observations are a matter of common experience; neither is here the place for considering other modes of exposure or the supposed influence which heretofore has obscured the etiology of tuberculosis to a certain extent, namely, the theory of a specific or more or less general predisposition. The relevant point is that, if we accept our present modes of diagnosis as reliable and agree that a positive cutaneous or other form of tuberculin test is an evidence of a preceding infection, regardless of its significance as to the extent to which this infection has been instrumental, or is likely to be so in the actual development of tuberculous disease, we must concede that the tuberculosis warfare with all its expenditures in effort and money has not been able to prevent infection under ordinary methods of exposure in families and elsewhere, and that the general rule requiring complete isolation of patients ill with infectious diseases holds good in tuberculosis, the same as it does in other communicable diseases.

It is needless to say that such an isolation is impossible in the case of tuberculous persons. This being the case it is unavoidable that not only tuberculosis-infections but also manifest cases of tuberculosis will recur in succeeding generations, and that therefore the disease is bound to perpetuate itself indefinitely, even though we were to increase our watchfulness and our general educational campaign, as long as isolation cannot be included in the prophylactic measures employed. As already intimated, the results from our present general efforts will, for the present and for years to come, probably show themselves mostly in the greater frequency with which a state of latency is maintained, either of the infection itself or of the tuberculous

served in the successive years to be 9 per cent; 20 per cent; 32 per cent; 52 per cent; 51 per cent; 61 per cent, 73 per cent; 71 per cent; 85 per cent; 93 per cent; 95 per cent; 94 per cent; 94 per cent;—from which they concluded that the practically universal tuberculization is accomplished at the age of 14 years, at least in the hospital population of Vienna, and that tuberculosis is in truth a children's disease, in the sense that the tuberculosis-infection is acquired almost invariably during childhood. Arranged according to age-periods, the steady increase of tuberculosis-infection is even more apparent and is found to be as follows:

I.	period—		1st year	0	per cent
II.	"	—	2nd "	9	" "
III.	"	— 3rd–	4th "	27	" "
IV.	"	— 5th–	6th "	51	" "
V.	"	— 7th–	10th "	71	" "
VI.	"	—11th–	14th "	94	" "

Leo Cohn[19] found in 283 children, all of tuberculous parentage, the cutaneous tuberculin test positive as follows:

2–5	years,	positive	in	66.66	per cent
5–7	"	"	"	77.5	" "
8–9	"	"	"	77.	" "
10–11	"	"	"	80.5	" "
12–13	"	"	"	89.9	" "
14	"	"	"	100.	" "

He believes that, in many of these children, negative results were due to general debility and cachexia, and that, in families in which the parents are ill with tuberculosis, all children are infected by the time they have reached their 14th year of life.

E. Feer[20] found among a clinical material of 2,000 children who were subjected to the cutaneous tuberculin test, that of 338 healthy children from tuberculous families, 76 or 22 per cent reacted to the test, while of 991 healthy children of non-tuberculous families only 114 or 11 per cent gave a positive reaction. He found further a family exposure to have existed in 86 out of 141 clinically tuberculous children, that is, in 61 per cent of the cases.

[19] Leo Cohn; Berl. klin. Woch. 191

[20] E. Feer; Beitr. z. Klin. d. Tub

processes which have occurred in consequence of the infection, until they heal out naturally and thus become obsolete in a constantly increasing number of cases. Further benefits will be secured in so far as a diagnosis is sought and made earlier, at a time before it can be confirmed by the demonstration of tubercle bacilli in the discharges of the patient. We can reasonably hope this result to show itself eventually in a more rapid downward progression of the death-rate than is given in the supposed natural rate of diminution, and indirectly by the beneficial influence of general hygiene, taking the term in its widest meaning, upon the mental and physical health and vigor of the people as a whole.

We feel therefore that the tuberculosis crusade is by no means a misdirected movement and that indeed it promises great benefits which time will show to exceed by far the cost, whatever this may have been. Although we do not look to the near future for a "natural" diminution of infection from extended and intensified prophylactic methods, as long as complete isolation is not feasible, or even for sufficiently striking changes in the direction of a lowering in the tuberculosis-mortality, except that the so-called natural rate of diminution will become greater (and the latter must come first before we can hope for the former), we nevertheless believe it to be an evident fact that in time the frequency of infection will decline together with a reduction in the morbidity and mortality.

Reibmayr and others expect such an outcome, not from general prophylactic efforts, but rather from the constantly increasing permeation of the race with tuberculosis, and from the sifting-out of the unfit through the disease, the survivors acquiring a greater resistance to the infection, which they transmit to their offspring.

In his interesting treatise on the marriage of the tuberculous and on its results, Reibmayr [21] claims that the disease is transmitted not only in the germ by contagion, but also as such by heredity, and in the latter case modified by a certain latency which is closely related to the, also transmitted, resistance or the power to struggle against the disease. As the disease has a national and international distribution, this re-

[21] A. REIBMAYR; Die Ehe Tuberkulöser und ihre Folgen. Leipzig & Wien, 1894.

sistance is gradually extended in the degree in which the non-resistant individuals are eliminated by death, and in which, more and more, only the resistant ones marry and procreate children. The introduction of new, that is, of non-resisting blood into a family may reduce this partial immunity which is greatest in those nations or races in which intermarriage is habitual (Jews). In the development of the national or the racial immunity, the elimination or sifting-out of the unfit is of great importance. Inbreeding promotes the development of the acquired and transmitted immunity in places where it is forced upon the people by geographical factors (islands, secluded valleys, political borders, differences in language), since in such cases a greater resistance to tuberculosis is observable than in countries in which "panmixia" prevails. This is true especially in the case of the Jews among whom the tuberculosis-mortality is lower, especially in childhood, and the duration of the disease is longer; the Jews frequently reach old age even if they are consumptive.

Therefore, according to this author, although tuberculosis may be spread by contagion, and although this is the primary mode of its extension and perpetuation, the hereditary transmission of the disease itself is of far greater importance for the ultimate outcome, for it brings about the permeation of the race with tuberculosis, and without this permeation the resistance to the disease cannot develop and cannot become a racial characteristic. The weaklings, the non-resistant ones die of the disease, the stronger survivors transmit a greater resistance to their offspring. This permeation of the race, promoted by intermarriage, will eventually lead to the victory of the race over the disease, at least in its fatal forms. In addition to the Jews whom the author cites as an instance of acquired and transmitted racial resistance, he refers to the inhabitants of England where, owing to the geographical isolation and the prevailing intermarriage, the permeation of the nation and the sifting-out of the unfit made greater progress early in the last century, where therefore a relative resistance developed, greater than in the nations of the continent, in consequence of which the tuberculosis-mortality was lowered decidedly beginning with the first third of the 19th century. An evidence of this relative resistance is seen in the greater frequency, over that of pulmonary tuberculosis, of lupus and of

lymph gland tuberculosis with marked tendency to fibrosis [22].

Concerning the acquired resistance of the Jews as a race, Maurice Fishberg [23] of New York denies the influence of intermarriage, showing that the Jews are by no means an unmixed race, and claiming that their relative protection is not owing to intermarriage. Fishberg attributes the latter to the fact that, for many centuries, the Jews have been city dwellers, accustomed to small, crowded and unhygienic quarters. They have long since passed through the more intense sifting-out process of the non-resistant and have paid the price of urbanization. While the incidence of tuberculosis is greater among them, and while the phthisical habitus is almost a racial characteristic, the acute and fatal forms of the disease are relatively infrequent; it is chronic, and the consumptive Jew lives far longer, resisting his disease better, than does the consumptive Gentile.

In support of the gradually acquired racial resistance to tuberculosis, which is transmitted to the progeny, Reibmayr cites the well-known fact that "new" races like the negro, the North American Indian and others, to whom tuberculosis was unknown on their first coming in contact with Caucasians, offered no resistance whatever and died in large numbers. Even to-day tuberculosis is a far more serious and fatal disease among such races than it is for Caucasians [24].

This view is also supported by Effertz [25] who relates that

[22] If we accept Reibmayr's ideas, we would expect the prevalence and the mortality of tuberculosis to be greater in the United States than in any other civilized country, because of the greater mixture of nations and races, and the practically unlimited panmixia. The people of the United States would therefore work out their salvation from tuberculosis only slowly, and not until conditions in this "melting-pot" of nations have become more settled and stable.

[23] Maurice Fishberg; Medical Record, 1908, LXXIV, 1077; and elsewhere.

[24] The relatively lower resistance of the negro and the North American Indian to tuberculosis, as compared with that enjoyed by the Caucasian, is undoubtedly emphasized by their unsanitary mode of living, their filthy habits, their indulgence in alcoholics, and by other factors of environment. For the Indian this is well illustrated in a study by Aleš Hrdlička, published in Bulletin No. 42, Smithsonian Institution, Bureau of American Ethnology; Washington, D. C., 1909.

[25] O. Effertz; Wien. klin. Woch. 1904, XVII, 129.

the tropical Indians in Central America acquire tuberculosis invariably in an acute form and succumb to it in the course of a few months. On the other hand, they are remarkably resistant to sepsis and syphilis, neither of which he ever observed in acute form. He concludes, that by centuries of permeation, they have acquired a racial immunity to these diseases, while this is not yet the case for tuberculosis which is relatively new to them. This disease, on the other hand, is resisted far more effectively by Europeans than syphilis which has been introduced to them, probably only a few centuries ago; and then it was far more severe than it is now. Effertz asserts that in all infectious diseases a racial immunity is acquired in course of time, owing to which the disease becomes more chronic and less severe.

While the last-named authors published their ideas only recently, the views of Reibmayr were promulgated over twenty years ago and were then far more startling than they appear now, in spite of the fact that, even before him, similar opinions had been expressed.

Häser [26] said that "the undoubted diminution, both in extent and in severity, of epidemic diseases, is due more particularly to two factors: to the removal and diminution of their immediate causes, and to the resistance to these diseases which increased with progressive civilization."

Rindfleisch [27] expressed the opinion that tuberculosis is an infectious disease to which the human genus has become adapted in a high degree. He believed that the disease is inherited like syphilis and that at one time it was similarly infectious, perhaps even more poisonous and malignant. By the great extension of its specific poison, the human race has become permeated with it, and in turn this has caused a certain degree of immunity.

The general evidence is to the effect that certain infectious diseases like leprosy, smallpox, typhus, cholera, or even pest and plague, have in the course of time not only become less frequent and more limited in their spread, but that their course has gradually become less severe; and these differences

[26] Heinrich Häser; Lehrbuch der Geschichte der Medicin und der epidemischen Krankheiten. 3te Bearbeitung. Jena. Bd. III. 1882, S. 956.

[27] Eduard Rindfleisch; Virchow's Archiv. 1881, LXXXV, 71.

could be observed before the advent of modern hygiene and quarantine, which in some countries are still crude or entirely lacking; and prior to the development of the modern therapeutic resources of treatment. Neither is the proposition untenable, that the individual immunity which follows an attack of these or other infectious diseases, may extend beyond the individual and be transmitted to the progeny. Such a theory is in keeping with the teachings of Darwin of the survival of the fittest who transmit their fitness to their offsprings; it agrees with Weismann's theory of the heredity of acquired characteristics, and it is given support by the result of more recent studies of the subject.

Since Meissen [28] as the first pointed out that heredity, in the sense that tuberculosis has existed in the ascendants, exerts no unfavorable influence upon the therapeutic results in consumptives, other observers have become interested in the problem, some of whose conclusions one of us cited in a paper on the subject [29] in 1907.

In this paper he was able to show that the so-called hereditary predisposition ("Belastung" or taint of Brehmer) referable to tuberculosis in the ascendants, not only does not cloud the prognosis, but that a benign course of the disease in the parents, as a rule, justifies the expectation *ceteris paribus* of a favorable course in the offspring. From a study of the records of 1415 cases of pulmonary tuberculosis treated at the Winyah Sanatorium, it appeared, in fact, that the chances of a favorable outcome were slightly better in those with a family history of tuberculosis than in those without such a history.

In experiments on chickens, Maffucci [30] found that chicks of tuberculous parentage, although infected more readily than those without such an hereditary "taint," were more resistant, their tuberculosis followed a more chronic course and showed a tendency to recovery.

Arthur Latham [31], physician to the Brompton Hospital for Diseases of the Chest, concluded from a study of the problem

[28] MEISSEN; Deut. Med. Ztg. 1885, No. 59. cf. H. WEICKER, Tuberkulose, Heilstätten, Dauererfolge, Leipzig, 1903, S. 37.

[29] K. v. RUCK; Amer. Jour. Med. Sciences, 1907, CXXXIV, 222.

[30] MAFFUCCI; abs. Centrlbl. f. Bakt. Ref. 1904, XXXIV, 708.

[31] ARTHUR LATHAM; The Lancet, 1908, II, 1512.

that probably the diminishing incidence and mortality of the disease may in part be due to a partial immunity inherited from tuberculous ancestors in whom the disease has been cured.

In our own investigations we have regularly been able to demonstrate specific agglutinins and, in many cases, specific amboceptors in the blood-serum of newborn children of tuberculous mothers, and other investigators have made similar observations in regard to other infectious diseases. So Schütz, cited by Schenk,[32] found diphtheritic antitoxin in the fetal blood serum, in the same, and sometimes in smaller amounts than those present in the maternal serum. Polano[33] found the same and he also destroyed the virulence of tetanus-toxin with the serum of children whose mothers had been immunized with tetanus-serum a few days *ante partum*. Fischl & Wunschheim[34] found in 70 per cent of their cases, fairly considerable quantities of diphtheria antitoxin.

Remlinger[35] could produce an antirabic immunity in young rabbits by immunizing the mother animals during gestation. He considers the process one of passive immunization, the effects of which are slight and transitory, not persisting beyond the sixth month of life. He found that antirabic immunity is not transmitted by nursing. In a case of Mosse & Daunic[36] the serum of a child born at term, whose mother had had typhoid fever in the sixth month of pregnancy, showed well-marked agglutinating power for typhoid bacilli. Hicks & French[37] collected from literature 21 cases of typhoid fever in pregnant women in most of whom premature labor had occurred. In 7 instances the fetal serum was capable of agglutinating typhoid bacilli. The authors raise the question whether the agglutinins are transmitted from the maternal blood, or whether they are produced in the fetal organism, that is, whether the immunization is a passive or an active one. According to Staubli's experiments[38] on guinea-pigs, the former appears to be the

[32] F. Schenk; Fol. Serol. 1909, II. 349.
[33] Polano; Centrlbl. f. Bakt. Ref. 1905, XXXVI, 518.
[34] Fischl & Wunschheim; Ztschr. f. Heilk. 1905, XVI. cf. F. Schenk, *loc. cit.*
[35] Remlinger; Ann. de l'Institut Pasteur. 1909, XXIII, 430.
[36] Mosse & Daunic; C. R. Soc. d. Biol. 1897, XLIX, 238.
[37] Hicks & French; The Lancet, 1905, I, 1491.
[38] Staubli; Centrlbl. f. Inn. Med. 1905, XXVI, 464.

case, and the same conclusion may be drawn from experiments on pregnant mice, made by Widal & Sicard [39] who found that the animals, injected subcutaneously with agglutinating typhoid serum, transmitted the power in one-half the strength to their young. In sucking guinea-pigs and cats, the maternal agglutinins were not transmitted. Schenk found in his investigations concerning the transmission of hemolysins and agglutinins, that certain antibodies (antihemolysins) were present in like amounts in the serum of the mother and her infant, while other substances were present in smaller amounts in the fetal than in the maternal blood.

In the blood serum of infants, in some instances born of clinically tuberculous mothers, agglutinins for tubercle bacilli were found by Hirigoyen [40], Jemma [41], F. Rosenberger [42], B. Salge [43], and in guinea-pigs, by F. Morelli [44]. The latter found the agglutinating power higher in the fetal than in the maternal serum and assumes that the former reacts more strongly to the toxic irritation than does the latter whose reactive powers are weakened by pregnancy.

Ferdinand Schenk found in the newborn young of tuberculous guinea-pigs treated with tubercle bacillus emulsion during pregnancy, the complement-binding substances qualitatively and quantitatively like those of the mother animals, but after a time they had diminished in the young. According to A. Fedali [45], however, amboceptors could not be found in the serum of the young of animals immunized by Maragliano's method, although small amounts of precipitins and agglutinins were present, and these amounts were larger in case the young were suckled by their mothers for considerable periods.

In a serological study, a few years ago, of blood specimens taken from the umbilical cords of over one hundred newborn infants, some interesting observations were made in our laboratory [46]. In the blood of six babies whose mothers had been

[39] WIDAL & SICARD; C. R. Soc. d. Biol. 1897, XLIX, 804.
[40] HIRIGOYEN; Gaz. Hebdom. d. Méd. et Chir. 1901 n.s. VI, 708.
[41] JEMMA; Centrlbl. f. Bakt. Ref. 1907, XXXIX, 86.
[42] F. ROSENBERGER; Centrlbl. f. Inn. Med. 1904, XXV, 665.
[43] B. SALGE; Schmidt's Jahrb. 1907, CCXCV, 75.
[44] F. MORELLI; Centrlbl. f. Bakt. Ref. 1912, LII, 441.
[45] A. FEDALI; Fol. Serol. 1911, VII, 199.
[46] KARL v. RUCK & H. J. ACHARD; Pediatrics, 1913, XXV, 742.

treated during pregnancy with Watery Extract of Tubercle Bacilli, agglutinins were found invariably in serum-dilutions of 1:50 (1 case) to as high as 1:225 (1 case). In one case specific amboceptors could be shown in but small amounts, in the other five cases they were found in serum-dilutions of 1:10 and higher; the opsonic index varied from 1.1 to 1.4.

In the sera of 30 other babies of mothers not treated by us, but with a history of tuberculosis, specific agglutinins were absent in 3 cases; they were present in the others in dilutions of 1:2 to 1:20, most often in dilutions of 1:5 and 1:10. Specific amboceptors could be shown, in smaller amounts, in 22 of the 30 specimens examined.

Since our blood-specimens were taken from the umbilical cord, our examinations would indicate that a transmission of specific antibodies can occur from the mother to the fetus through the placental circulation. If this is the case, there appears to us no valid reason why the specific toxins or their split products can not also reach the fetal circulation if they are present in the blood of the mother, nor why in such a case the fetal organism should not react and itself produce specific amboceptors, i. e., acquire an active immunity against the particular toxic fraction or fractions of the tubercle bacilli; the fact, that we found specific amboceptors on repeated examinations in non-tuberculous and not treated children up to ages of four and six years, supports this view. If split products also pass over from the mother, and the fetal blood does not possess sufficient specific amboceptors to reduce them promptly, they can produce a general reaction in the fetus, the same as in the mother, and a toxemia resulting in this manner may be responsible for the often observed death of the fetus and for the frequent abortions which occur in pregnant women who suffer from active tuberculosis.

The occurrence of passive or of active immunity, or of both, in the fetus accounts for the infrequency of manifest tuberculosis in the newborn and during the early months of life, as well as for the observations of tubercle bacilli without tubercle in the fetal blood or organs, which have been recorded in literature. The toxins which pass the placental circulation can cause varying degrees of specific resistance in the fetal organism the same as does their artificial administration after birth, and the

variations of the resultant immunity will become more intelligible if we are correct in our position that, in order for the specific resistance to become complete, the several specific bacillary body-substances must all be represented in the organism by their related specific amboceptors. In this light we are then not surprised that the fetal organism may possess varying degrees of specific resistance or that this may be entirely lacking in it.

Like in the mother, the active immunity acquired by the fetus need not be maximal or complete in the sense that it is present against all the body-proteids or lipoids of the tubercle bacillus. The acquired specific resistance of either mother or fetus is represented by bacteriolytic amboceptors which correspond with the toxic substance which caused their production. The amboceptors appeared to correspond quantitatively and qualitatively also in our examinations of blood-specimens taken from the mother and from the newborn infant at the same time.

We have stated our reasons on other occasions for believing that the spontaneously acquired immunity against the tubercle bacillus is not evolved against its specific secretions and body-constituents simultaneously, and we again point out that its development must occur gradually and step by step, first against those toxins which the bacilli produce in their living state, and next against the several constituents of their bodies according to their solubility. In order that a spontaneous immunity may be acquired against any of the bacillary body-constituents, the bacilli must first die and become disintegrated; on account of their great resistance to solvents and of their enclosure in non-vascular tubercles this immunity can be but slight before the tubercles have become caseous and have softened, after which the more or less liquefied material is available for absorption.

We may perhaps elucidate the subject of the spontaneous acquirement, by the fetus, of passive or of active immunity or of both against the bacillus of tuberculosis, by applying our theory hypothetically. Taking for example the case of a non-tuberculous mother, it would be found that the blood of the newborn infant contains no specific antibodies against tubercle bacilli or their products.

Assuming that the mother has acquired tuberculosis and that her disease is in that early stage in which destructive changes

have not yet occurred, it can happen that a local immunity has developed in and peripherally to the lesions, sufficient to hold the disease in check, because the cells of the local lesions may have been stimulated by toxic substances FROM THE BODIES OF TUBERCLE BACILLI, which, being available in only very minute quantity at so early a period, have not been absorbed in sufficient amount, or none may have been absorbed at all, to cause a general immunizing effect by which the specific amboceptors would occur free in her blood. The mother's general immunity may thus be limited and represented by specific amboceptors against the secretion- or metabolic toxins of tubercle bacilli, and her local immunity may include a limited amount of amboceptors which are specific for one or several of the body-constituents, i. e., of the endotoxins. In such a case the amboceptors, which the mother could transmit to the fetus in appreciable quantity, would be those which she produces in larger amounts, and which are present in her blood, and these would be active against the exotoxins, i. e., against the secretion-products which the tubercle bacilli elaborate in their living state. The fetus can thus acquire a certain degree of transmitted passive immunity against the secretion-products of living tubercle bacilli; but it has not acquired an active immunity against them, and when the supply of the specific amboceptors from the mother ceases, they soon disappear from the blood of the fetus. Although the blood of the newborn child contains amboceptors transmitted from the mother, the infant does not react to the cutaneous test with the related toxin, because the amboceptors are diffused in its body fluids and cannot accumulate in sufficient amount, at the site where the test was applied, to reduce and split the toxin before it is bound by local cell-receptors, or is absorbed.

Assuming, however, that during the course of pregnancy, the mother's local immunity became insufficient to hold the disease in check, the results to herself as well as to the fetus or newborn child would differ. Through changes in the maternal cellular metabolism, or through changes in the local circulation of the tuberculous lesions, or through other causes, the growth-energy of the tubercle bacilli in the lesion can increase; the local specific amboceptors for their body-substances, if present, are then inadequate, and in such a case new tissue is invaded by living

bacilli; consequently there is a corresponding increase of the bacillary secretions or excretions. The absorption of body-substances which are essential for the production of amboceptors against the bacilli or their endotoxins would, however, not be increased and no amboceptors against them would be available in the newly invaded tissue.

In this case the mother's general immunity against the toxic substance which the bacilli secrete, may increase enough to bind and reduce the toxin which she absorbs in greater quantity; but if the latter is in excess, split products will occur in her blood, to which she responds with more or less fever. The whole toxin and the split products of partially reduced toxin can then also pass to the fetus through the placental circulation and become bound by cell-receptors of the fetal tissue. The fetus thus acquires an active immunity against the particular toxin, the increase and final degree of which depend upon the continuance or repeated presence of unreduced toxin in the mother's blood, and upon the quantity which reaches the fetus at any one time. The acquired immunity of the fetus may become ample to bind and completely reduce new accesses of toxin, or it may fall short because, by reason of the increase of the mother's immunity against the same toxin, none may thereafter reach the fetus; or, if small amounts did pass the placenta, they would be promptly reduced by free amboceptors which the fetal tissues can now supply to its own blood.

The tissues of the fetus or of the newborn infant have then acquired a variable degree of general active immunity against the particular toxins, and can thereafter continue to produce free specific amboceptors for them. In case a tuberculin test is made, these amboceptors increase locally, by being detached from previously sensitized cells, and the occurrence of a reaction depends, on the one hand, upon the degree of the general active immunity as manifested locally, and, on the other, upon the amount of the identical specific toxin which was present in the test fluid. Such an infant possesses an active immunity against the secretion-products of tubercle bacilli, but not against its body-substances.

Assuming that the mother's disease had advanced further before pregnancy occurred, or that caseous softening and absorption of the more or less disintegrated tuberculous tissues takes

place during pregnancy, the results to her and to the fetus can again differ and the differences can now become very numerous and complex.

In this stage of the mother's disease, she has absorbed or is absorbing specific body-substances of the tubercle bacilli, either in the form of whole bacilli or their fragments and granules or in a state of further disintegration, and of their several proteid and lipoid constituents in solution. The absorbed bacilli may be living and more or less virulent, or they may be avirulent or dead. The amount of the absorbed bacillary body-substance in any of its physical states may differ quantitatively as well as qualitatively. The mother may previously have acquired, or may now acquire, a certain amount of general immunity against one or all of the bacillary body-substances, or she may not possess enough or any specific amboceptors for their prompt parenteral reduction to a simple, elementary, non-toxic form. The maternal blood can thus contain unchanged or split products of one or several of the bacillary endotoxins, and it may also contain related specific amboceptors in varying amounts. The content of the maternal blood, in toxins and specific amboceptors, may vary from day to day, and their transmission to the fetus is subject to like variations.

It would require almost unlimited space to consider all the possible changes and results, as they would become manifest in toxemia and in immunizing responses on the part of the mother and of the fetus; the reader can, however, readily appreciate the most important responses in either of them. In case the mother absorbs bacillary body-substances from her tuberculous lesions, the fetus may receive them from the maternal blood, unchanged or in the form of split products, or both, and while it thus develops an active immunity against them, it may likewise participate in the mother's toxemia, in case she possesses specific amboceptors but in an insufficient amount to reduce the related toxin fully. If it is further borne in mind that the bacillary body-constituents absorbed by the mother, or the portion of them that passes the placental circulation, may or may not represent all of the several body-constituents of the tubercle bacillus, it will be appreciated that the mother or the fetus may acquire either a complete or only a partial immunity, and that both can vary according to the degree of specific stimulation, and to the

frequency of its repetition to which the tissue cells of either of them have been subjected.

In case living virulent tubercle bacilli have also reached the maternal circulation during the destructive stage of disease, and if not all specific amboceptors are present which are necessary to cause their destruction, their virulence either may become reduced, it may remain uninfluenced, or it may even be increased if the aggressin-theory of Bail is correct in the sense that the living tubercle bacillus can defend itself against injury. Accordingly the mother may or may not suffer an extension or new localization of tubercles in other organs and parts.

Although the permeability of the placenta by corpuscular elements is not generally admitted, that of mucous membranes is conceded by most students. If we accept it for the placenta, the fetus is subjected to intra-uterine infection if virulent tubercle bacilli reach its tissues from the maternal blood.

Inasmuch as the fetus may previously have received specific amboceptors from the maternal blood, or the fetal cells may previously have been stimulated or may now receive such stimulation for the first time through bacillary body-substances that have passed the placental circulation, its tissues may at once be capable, or they may speedily acquire the power of offering a sufficient defense, to prevent or to check the growth-energy of the bacilli. The bacilli would then be destroyed or would be injured sufficiently to remain latent, and anatomical lesions would not occur. In the prolonged absence of such a defense, or in case the infection of the fetus has been massive, as it is apt to be if the placenta is itself the seat of tuberculosis, tubercles would form in the fetal tissues, and the time of such an occurrence, the number of tubercles, as also their destiny would be influenced by and proportionate to the number of bacilli and their virulence.

The passage, by tubercle bacilli, through normal tissue like the mucosa is never rapid and massive; it is more probable that only few individual bacilli will thus pass and that, in case of the placental transmission of tubercle bacilli, their passage would be similarly limited. This would afford the fetal organism a better opportunity of increasing its specific amboceptors and of limiting the pathogenic action of the bacilli by opposing or inhibiting their growth and multiplication.

In tuberculous mothers, especially those who, during gestation, may be properly subjected to specific treatment, the constituents of the specific preparation, whatever they may happen to represent, and the related specific amboceptors in the blood alternate, and a like alternation must obtain in the fetus. The specific antibodies which are present in the newborn child are then in part the products of its own cells, on account of active immunization, and in part the products of the maternal cells. The function acquired by the fetal organism, of producing free amboceptors for the related specific products of tubercle bacilli, continues active and potential for a long period of time, whereas the transmitted amboceptors disappear, as in the case of their artificial administration for the purpose of passive immunization.

The increasing frequency with which reliable observations indicate that tubercle bacilli are often present in the blood of tuberculous subjects, the observation of H. Much and others, which we can confirm, that failure to demonstrate tubercle bacilli with the carbolfuchsin method of staining does not exclude their presence and that certain forms of them can still be demonstrated by Gram's method, is of additional significance in the study of the congenital transmission of tuberculosis-infection, and if the latter is considered in the light of the fact that varying degrees of active immunization can obtain during fetal life, we must necessarily reconsider the general denial of the importance of v. Baumgarten's studies and investigations. It is well known that Professor v. Baumgarten attaches great importance to the relation of congenital infection to the occurrence of tuberculosis in later years, and the number of those who are more recently adjusting their attitude in his support, is steadily increasing. The objections heretofore urged most frequently to v. Baumgarten's position, namely, that it postulates so long a period of latency between infection and the occurrence of clinically manifest tuberculosis, fall to the ground if we are correct in our belief that the fetus may acquire an active immunity against the tubercle bacillus and its toxins, because in such a case a relative amount of specific resistance may be continued for the intervening period during which the power of tubercle bacilli for reproduction and virulence may be checked and remain latent, and a sufficiency of bacteriolytic power, acquired by the fetal organism, can account for in-

stances in which the intra-uterine or a subsequent infection was not followed by disease.

Our observation of specific amboceptors for tubercle bacilli and their several constituents, in the sera of children at birth and in two of them up to four and six years thereafter, the continued exposure to infection, the negative results of cutaneous tests after v. Pirquet's method, and the bactericidal power of their sera upon virulent tubercle bacilli, all tend to confirm the view that an active and complete immunity obtained in them prior to their birth and that it was probably augmented by the treatment which their mothers received during gestation with increasing and eventually large doses of the Watery Extract of Tubercle Bacilli.

The difference in the antibody-content of the sera, between them and other children born of tuberculous mothers who had not been treated specifically, is only quantitative, and while among the children of non-treated tuberculous mothers we found the antibodies to be, as a rule, only partial, often in small amounts, and although in some cases we found none at all, these findings fully support the view that an active immunity can be acquired by the fetal organism through the transit of the related specific antigen from the mother's blood to that of the fetus, since the children of treated mothers showed it in greater amount, including amboceptors for all antigens with which their sera were tested.

We have already referred to Reibmayr's theory which is of interest in connection with the subject of the congenital origin of tuberculosis. In regard to the latter, it would, however, be erroneous to assume that v. Baumgarten denies the occurrence of epidemiologic infections, or that he underestimates their importance, although he believes them to occur less frequently than is generally believed to obtain at the present time. Opinions in regard to the importance of one or the other modes of infection can only relate to the question of frequency, and however this may be found to be, we are not in a position to relax our preventive measures against the exposure of children, or even against the exposure of adults, because the children may have acquired a prenatal infection, or because most infections appear to have been contracted before the individuals have reached adult age.

In this respect it is important to bear in mind that it is possible for a superinfection to occur at any age and that this may become the deciding factor in the establishment of manifest disease, as v. Behring believes it to be.

We were forcibly reminded that this question is an important one, by our observation of the results of superinfection in laboratory animals, which will be considered further in a subsequent chapter. Here we wish to point out that, in small animals like guinea-pigs, superinfection proved most disastrous in our experience and that we could not confirm Koch's statement according to which a second infection fails in this class of animals, or that it remains confined to the local infection site which heals spontaneously after breaking down. We found, on the contrary, that a superinfection may kill a guinea-pig even in the course of a few days, that the primary infection becomes greatly intensified in animals which live longer, and that the second infection may cause the rapid evolution of new miliary tubercles in internal organs, and cause focal necrosis.

According to our observations, the course of tuberculosis in guinea-pigs does not differ in any important essential from that in the human subject. We find that it can assume any type, from the acute miliary to an extremely chronic one which resembles the so-called fibroid phthisis of the human subject with coexisting signs and multiple pulmonary cavities and, in at least one case observed by us, the tuberculous lesions consisted only of fibrous tissue. We have had occasion likewise to witness a remarkably detrimental influence from the occurrence of associated infections with other bacteria upon the course of the disease in guinea-pigs, for when such an infection was present, the course of our infection with tubercle bacilli, as a rule, was influenced unfavorably.

These observations we believe to correspond in all respects so closely with those made in the course of human tuberculosis, that we may well inquire whether or not there is in fact an exception in the case of virulent superinfection in tuberculous children or adults, and whether the effects of superinfection in them are not after all only relative and appear less severe in the human subject or in larger animals, by reason of differences in general resistance and in their relative size.

In the course of our experimental studies, our attention has

been directed also to a possible adverse influence of a slight degree of acquired immunity upon the course of artificial infection. Although such large numbers of tubercle bacilli as must be used in artificial infections in order to assure the uniform occurrence of tuberculous disease in comparative experiments, are not at all likely ever to invade the human organism at one time, and the mode of infection differs in that the bacilli are not subject to the mechanical defensive provisions against their entrance into the tissues, but are introduced directly in large numbers at the site of infection, the subject nevertheless became of interest to us. Our observations were exceptional and our opposite results in other experiments of the same kind suggest a coincidence. The facts, which we noted as long as twenty years ago and on various occasions since, were that certain animals, which were infected after a course of specific treatment with various products of tubercle bacilli, appeared to lose weight, showed enlarged regional lymph glands and caseous changes at the infection sites, died of tuberculosis earlier, and on autopsy showed more extensive and severe types of disease of internal organs than we observed in controls. While such results were noted with increasing frequency in animals which had acquired pseudo-tuberculosis, they were noted also in instances in which it was impossible to show that pseudo or other spontaneous infections had either preceded or followed ours. We found the lesions to be typically tuberculous, and the most painstaking bacteriological studies failed to demonstrate the presence of other microörganisms in the diseased or normal tissues including the blood. In sections stained for tubercle bacilli, these were so numerous at times as to attract attention. No pseudo or other infections being demonstrable and the conditions of infection having been identical in both the principals and the controls, an explanation of the anomalous observations had to be sought in the animals themselves. Having failed to find one and having noted a like behavior repeatedly, we have naturally thought of BAIL'S THEORY OF AGGRESSINS, by which our observations would become intelligible, in that it permits the assumption that the tubercle bacilli, which had been introduced, were subjected to the action of certain specific antibodies of the partially immunized animal, or of antibodies which were contained in the serum with which they

were incubated. Although insufficient to destroy the tubercle bacilli, these specific antibodies could nevertheless tend to inflict injury upon them. This being the case, the tubercle bacilli produced what Bail calls aggressins, for their protection against the action of the antibodies, and these aggressins destroyed or neutralized them or prevented their action by causing a negative chemotaxis. This would constitute a specific resistance acquired by the bacillus, very similar to that which is acquired by the animal organism, there being, in either case, an increase of a cell-function for a protective purpose.

If this were the true explanation, it would mean that under certain conditions the existence of a slight or partial degree of immunity may become equivalent to a specific predisposition, by arousing the defensive function of the tubercle bacillus and thus increasing its virulence. The interactions and reactions which follow would represent the usual struggle for ascendency between the parasite and the host, which could not arise in the presence of a full degree of immunity in the host-organism, because its tissues and fluids would possess ample bacteriolytic power to kill and to cause the disintegration of the tubercle bacilli before they could develop their latent function of defense.

The literature upon experimental immunization against the bacillus of tuberculosis contains numerous observations corresponding with the facts which we observed and have mentioned, in that the treated animals sometimes succumbed very soon to artificial infection and before the controls died of tuberculosis. While we do not at all wish to be understood as advancing a theory of specific predisposition, or of supporting those who still adhere to it, such a relation is at least thinkable.

In the meanwhile we remain aware of the fact that, if our theory or a similar one were found to be true, it would be necessary to reconsider certain other theories in the etiology and in the specific treatment of tuberculosis, and to examine again the facts upon which they are based. While the study of the above and of many other questions in tuberculosis is important, it is so chiefly for the elucidation of the true relation of facts observed in the clinic and in the laboratory, and for the correction of our current theories which have been built upon them.

In continuing our efforts to find the truth, we may hope to approach it more closely or actually to demonstrate it; but we

are also liable to replace an existing erroneous theory by another one equally so, or indeed to abandon a true interpretation for a wrong one. Pending the consideration of such moot questions, the clinician must hold fast to his experiences at the bedside and remain undisturbed, while he continues to be an interested spectator of the struggle of investigators to find the truth. There should be no partizan attitude on his part or on the part of him who has the opportunity to conduct such investigations, with a view of upholding any particular theory, even though it has originated with himself. The end sought being the prevention and cure of tuberculous disease, the clinician must ever remain the umpire and, in order to render a true verdict in the settlement of any of the many moot questions, he, as well as those who study them experimentally, must observe and examine all evidences with an open mind.

The acquirement of natural immunity by the human race, against tuberculosis, in accordance with the theories of Reibmayr and others, would no doubt require many centuries, during the earlier ones of which the mortality, although diminishing, would continue to be high. While the antituberculosis movement offers better prospects as regards time that this mortality will decrease at a more rapid rate and thereby lessen the number of infections as well, its efforts to eliminate the opportunities for exposure to infection would, according to Reibmayr's theory, tend to lessen the hereditarily transmitted specific resistance. In any case, a long and hard struggle is still before us, even though we continue the general campaign in increasing intensity.

All students of the subject must therefore realize that the only prospect for an earlier success lies either in the discovery of a method of treatment which will cure tuberculosis with certainty in its advanced stages, or in a reliable and practical method of protective vaccination, which will prevent the development and evolution of tuberculous disease after infection has taken place.

CHAPTER II

IMMUNITY IN INFECTIOUS DISEASES

Broadly speaking it may be said that *infection* is the invasion of the living body by a pathogenic microörganism, and the ability of the invader to multiply and functionate within the cell-complexes of the host which, through these foreign life-processes within it, is disturbed in its own normal functions, reacts to the invasion, and does or does not become diseased, accordingly as its defensive reaction is not or is successful. The invading microörganisms (bacteria, protozoa, etc.) thus assume the rôle of parasites.

A sharp distinction between pathogenic and non-pathogenic microörganisms or between parasites and saprophytes cannot be drawn, because their power to produce disease in the organism of the invaded host is relative and depends upon various factors. Human and animal organisms constantly harbor a great many bacteria upon their cutaneous and mucous surfaces, which ordinarily cause no infection because they do not invade, and multiply in the physiological interior of the body; and even if they do, they are usually destroyed promptly. Under certain conditions, however, for instance, cell-death, they may enter the tissues and become pathogenic for them.

Bacteria, which are capable of producing disease in their host, are chiefly of two kinds. The first remain at or near the point of entrance, where they secrete poisonous substances which are absorbed and are carried into the interior of the body in which they produce disease by intoxication, as is the case in diphtheria and tetanus. The second localize and multiply within the body and may be carried to other parts by the circulation. They cause disease, mechanically, through the tissue-reaction which they set up (for instance, tubercle, typhoid ulcer), or by embolism in the capillaries, or by poisonous substances mainly derived from their bodies after their death and disintegration. The degree in which they do this constitutes

what is designated as *virulence*, whereas the opposing efforts on the part of the organism are spoken of as *resistance*.

Virulence of disease-producing microörganisms, or their power to cause actual disease, and the manifestations and course of the latter vary in degree, not only for the particular species of animal, but also for individuals of the same species and, in the latter case, according to the localization where the microorganisms have established themselves. Virulence varies again according to the previous conditions under which the microorganisms have grown and perpetuated themselves and according to the conditions to which they are subjected during the time intervening after leaving, or being discharged from the old, and gaining entrance into the new host.

The accomplishment of the infection, in the sense that it is followed by evidence of disease, appears to be governed also by the number of the bacteria which gain entrance, although in theory this should influence only the rapidity with which evidence of disease follows; unless a quantitative relation may be assumed to exist, in the sense that the resistance yields to greater numbers of bacteria, when it is effective against smaller ones, as it seems to be the case in experimental infection. It must, however, be taken into consideration that, even in experimental infections, it is not possible to know the number of viable bacteria which are present in the tissues, secretions, or in the suspensions made from pure cultures, which are used for the infection, and that nothing at all can be known, in the case of epidemiological infection, of the number of bacteria that prove effective. For practical purposes, however, we are justified in accepting a numerical relation in both cases.

According to the supposed massiveness, intensity or virulence of an infection, to the rapidity with which the bacteria multiply, and to the degree of opposition with which the infection is met by the host-organism, the results observed in the course of the infectious disease are differentiated by most authors as malignant or fulminating, acute and chronic.

There is a *period of incubation* between the invasion of the organism by microörganisms and the successful establishment of infection leading to the related infectious disease, which represents the time necessary for growth, distribution and accumulation of the bacteria or their toxic products and which, strictly

speaking, establishes and limits the infection itself. Von Pirquet & Schick [1] suggest that this time may correspond roughly with the interval during which the subject is becoming "allergic." H. Koch [2] has found in three observations of known tuberculous infection in children, that a positive v. Pirquet reaction could be elicited about seven weeks after the bacterial invasion.

The period of incubation can be determined only if the time of exposure is known, and also the time when the virus actually gained entrance into the tissues, conditions which ordinarily are complied with only in the experiment, while in spontaneous infections the time of incubation can be reckoned only from the time of a known exposure. The period of incubation has been found to be fairly uniform in acute and in some chronic infectious diseases.

In tuberculosis we find that, in the experiment animal, the period of incubation appears to be intimately related to the virulence and massiveness of the infection, as well as to individual peculiarities of the infected animal, its age and size, etc., and to other, unknown, factors which appear to exert an influence. It frequently happens after experimental infection, when conditions are equal in all respects, as far as we can judge, that some animals show symptoms of tuberculosis, or even die of it, at a time when others as yet appear to be entirely uninfluenced and live much longer, often twice as long as the first. The supposed non-resistance of guinea-pigs to tuberculosis-infection therefore is only relative and, in certain individual animals, it may occasionally be changed even to a complete resistance, if the virulence of the culture of tubercle bacilli used for infection is moderate or slight. Several observations have come to our notice in which guinea-pigs resisted the infection completely, when other animals of the same group succumbed under identical conditions of age, sex, weight and subsequent care, and under identically the same mode of infection made at the same time, with like doses from the same emulsion of a known virulent culture. This difference in the response to infection appears to be still greater in larger animals.

In man it is, of course, rarely possible to determine the time

[1] C. v. Pirquet & B. Schick; Wien. klin. Woch. 1903, XVI, 758 and 1244.
[2] H. Koch; Wien. klin. Woch. 1915, XXVIII, 77.

of an infection; even that of exposure often cannot be ascertained definitely, and nothing at all is known of the number or the virulence of tubercle bacilli that may have gained entrance. If then we speak at all of an incubation period, represented by the time between the access of tubercle bacilli to the tissues and the development of symptoms, it is with the understanding that its duration cannot be determined exactly; and if we attempt to estimate it from the time of a known exposure, it is impossible, as a rule, to exclude preceding or subsequent exposures; nor would it be proper to base our reckoning upon the first positive result of a v. Pirquet test, because this does not prove that the infection has been established in the sense that it has caused tubercle-formation; indeed, symptoms and signs of disease may be deferred for months and even years, or they need never occur, after a positive tuberculin test has been observed. [3]

RESISTANCE OR NATURAL IMMUNITY

The Romans originally designated persons or nations as *immune* who were exempt from the necessity of paying taxes or tribute, and they themselves already extended the application of the term to those persons who did not pay tribute to certain diseases and to harmful influences in general, to which others fell victims or succumbed. We understand by immunity, in a somewhat restricted sense, the freedom or exemption from climatic or sporadic influences, more particularly from infectious or epidemic diseases, as it is observed in species, races, and individuals. Immunity is a term denoting an abstract idea rather than a concrete fact and, although one may speak of absolute immunity, this can mean only an immunity which

[3] According to Much & Leschke [Beitr. z. Klin. d. Tuberkulose, 1914, XXXI, 335] a positive v. Pirquet reaction may be produced after the passive transmission of immunity, entirely without tubercle formation. Similar observations have been made by us and by other investigators, for instance by Jousset [Bull. Acad. d. Méd.; 1915, 3e sér.; LXXIII, 635.—abstr. Journ. A. M. A., 1915, LXV, 284]. These observations tend to show that a positive v. Pirquet test indicates the reactibility or hypersensibility of the organism to a certain bacterial substance, but not necessarily an infection with the related bacterium.

is effective under ordinary conditions, for even the supposedly absolute immunity of species, as for instance that of chickens to anthrax or to tetanus, can be overcome.

An immunity to infectious diseases is not enjoyed only by individuals, but is a characteristic of *species* in animals. It is well known that certain infectious diseases, common in man, are not observed in animals, as for instance syphilis, typhoid fever, cholera, scarlet fever, measles, etc., and that man is practically exempt from others, like rinderpest, distemper of horses, pleuro-pneumonia of cattle, goats and sheep; whereas both man and animals are liable to some infectious diseases like tuberculosis and anthrax.

Similar observations could be cited from the vegetable kingdom, showing that certain species of plants are subject to parasitic diseases from which others are free.

Coldblooded animals are almost absolutely immune to infection with bacteria that are virulent for man, and vice versa; but exceptions have seemingly been observed, like in the two cases of spontaneous pulmonary phthisis and pulmonary tuberculosis in turtles described by Friedmann in 1903, both of which were probably due to infection with tubercle bacilli of human origin; other authors have succeeded in producing experimental tuberculosis in coldblooded animals with human and bovine bacilli.

Such observations are, however, not made under ordinary conditions and, in order to overcome the resistance of species, special modes of infection are required or the animals are made receptive by exposure to cold, hunger, change of temperature-conditions, and by other influences which alter their normal vital processes.

An increased resistance appears to be characteristic for certain *races* of the same species, against a given infection, and is illustrated, for instance, by the resistance of Yorkshire hogs to swine-erysipelas and that of buffalo calves to artificial infection with large amounts of bovine tubercle bacilli; also in the comparative resistance of Algerian sheep to anthrax and to cow-pox. A like racial resistance was believed to exist in certain races of man to infectious or contagious diseases prevalent among Caucasians, especially tuberculosis and phthisis; but the assumption proved to be unfounded with more extensive obser-

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vation. In fact, so-called *new* races were found to be peculiarly non-resistant to such diseases after they had once been exposed to infection, and still more after they had proved apt pupils in acquiring the evil and undesirable offshoots of civilization, by which the benefits of their former natural modes of life were lost and their natural resistance was impaired. In those instances in which a racial immunity seemingly exists, for instance, in the different susceptibility of Europeans, and of aborigines of various countries to bubonic plague, to cholera, etc., the cause may be a hereditary transmission of specific resistance; but the observations of this kind may also be explained by differences in the hygienic and other conditions of life, since the better situated classes or castes of natives are likewise affected less by such diseases than are the poorer portions of the population. In many cases an apparent natural resistance is in reality acquired, for instance that to malaria and to yellow fever in the negro.

The natural resistance may be subject to considerable variations in *individuals* of the species. We have already referred to our observations in regard to differences in the response to artificial infection with tubercle bacilli in the guinea-pig. That such differences in resistance are liable to occur also in the natural acquirement of the disease, we had the opportunity of witnessing in animals which had acquired tuberculosis spontaneously. In ONE such animal the evidences of healing were so uniformly marked, in the form of fibrous changes in every tuberculous lesion found on autopsy, that death could not be attributed directly to tuberculosis, but appeared to have been due to a loss of function on the part of the involved organs, the degree of which would have been insufficient to cause serious disturbance in a large animal with a like extent of fibrosis.

Differences in resistance or in susceptibility to like harmful influences are manifest more particularly in man, it being a general observation in every epidemic that many persons prove resistant to scarlet fever, smallpox, typhoid fever, influenza, etc., without having passed through previous attacks of these diseases, and that even in severe cholera or plague epidemics only a portion of the population is affected, although practically every one appears to have been exposed in an equal degree.

Similar observations are made in epidemics in animals, for instance, in foot-and-mouth disease.

The resistance or immunity which is sufficient to protect certain species of animals, including man, against parasitic injuries that ordinarily are communicated by contact, differs from the immunity which is acquired by inoculation or by passing through an attack of a certain disease, or by protective vaccination against it, and has been designated as *Natural Resistance*, or *Natural Immunity*. It was assumed to exist not only in classes but also in individuals, in so far as they were found to resist infection in epidemics or in other indubitable exposures.

If it is considered that most bacterial life is ubiquitous and that some pathogenic bacteria are distributed in nature so uniformly that contact with them cannot be avoided by the animal and human organisms; if it is realized further that these microörganisms follow the natural law of seeking sustenance wherever it may be found, and of overcoming hostile influences wherever this is possible, it is easy to appreciate that the organism would speedily succumb to the aggression of its multifarious bacterial enemies, if it were without the means of resisting their invasion. By their presence and activity a balance is maintained between macro- and microörganic life, neither of which obtains a lasting ascendency over the other, except under unusual, that is to say, abnormal conditions. The ability to resist bacterial action is dependent primarily upon the continuation of life, for immediately after death, either of the whole body, or of a part owing to the permanent localized suspension of the blood supply, certain saprophytic bacteria (putrefactive bacteria) are able to multiply and to exert their activity.

During life, the harmful action of microörganisms depends largely upon the efficiency of the resisting forces which the organism possesses normally, or which it is capable of developing in the event of infection. These resisting forces are not equally potent against all varieties of infections; the same organism may possess an absolute immunity against certain microorganisms and it may, at the same time, be very susceptible to infection with others. Between the extremes, all degrees of relative resistance exist, which determine the predisposition to

the acquirement of an infectious disease, or the immunity against it.

The access of bacteria into the living organism is resisted by several defensive provisions. The outer lines of defense include the integument, and the skin is usually sufficiently impervious to prevent the entrance of bacteria, so that these can cause infection only if a solution exists in its continuity, at least of the epidermis. The mucous membranes are not absolutely impermeable; even if they are not injured, they often form ports of entry for various bacteria. Except for genital and rectal infections, the bacteria are introduced mainly with the air, water and food, and they may determine either respiratory, gastro-intestinal or generalized infections by lodgment in the respiratory or digestive tracts. In either case the surface-discharges and secretions, and the epithelial covering of the mucous membranes (especially those of the pavement- and ciliated varieties), the natural peristalsis, the normal secretions, and the admixture of fluids and solids in the digestive tract, tend to prevent the entrance and localization of bacteria mechanically, or then to inhibit their multiplication.

After the outer lines of defense are overcome, the invading bacteria are further opposed by an internal line, consisting of mobile and sessile cells which attempt to remove and to destroy them by their phagocytic action. The bacteria are then subjected also to the normal bactericidal action of the blood and tissue-fluids, of the secretions of certain excretory glands, and of the lymphoid tissue. There is, however, evidence that the bacteria may increase their offensive power, by multiplication and by special and specific provisions, for the purpose of protecting themselves against the action of the internal defensive provisions of the organism, namely, by capsule- and spore-formation, and by poisonous secretions. We have already referred to the latter to which Bail [4] has devoted special attention and concerning which he has published most interesting observations. His theory of the *aggressins*, as defensive secretion-products of the tubercle bacillus, is well known and is supported by similar observations, in our country, for instance, by those

[4] Oskar Bail; Wien. klin. Woch.; 1905, XVIII, 211; 547; 1212.—Arch. f. Hyg.; 1915, LII, 272; LIII, 302.—Fol. Serol.; 1911, VII, 119, and elsewhere.

of Welch [5] and by those of Rosenow [6] who calls them *virulins*, that is, the substances upon which virulence would seem to depend. In like manner, or in a similar manner, the organism of the host is able to respond to infection by additional defensive provisions the activation of which results in reaction-products in the form of antibacterial substances which are specific against the related pathogenic microörganisms.

The outcome of the struggle between the invading bacteria and the invaded host may be a complete victory on either side; but it may also remain undecided, an apparent balance being maintained for a time between the offensive and the defensive forces, until one or the other gains the ascendency. In chronic affections like tuberculosis, the result often is only a partial one; the bacilli are not destroyed but become surrounded and imprisoned by a peripheral, fibrous enclosure of the territory which they occupy, formed by the activity and proliferation of cells and by their organization into connective tissue which becomes condensed and forms a fibrous capsule. Such an enclosure prevents the escape of the prisoners, if it is complete, and they are subjected, within it, to a tedious process of starvation; but they are likely to escape as long as their destruction is not fully accomplished. On the other hand, the fibrous capsule prevents, to a certain degree, the access to the bacteria of the tissue-fluids which may carry specific antibacterial substances and may therefore injure those bacteria with which they come in contact.

Predisposition

The observation that certain persons yielded or succumbed to certain infections, while others resisted them, forced the theory of direct heredity or of inherited predisposition to disease upon the minds of observers. The direct inheritance of a disease, or the transmission of peculiar humoral and structural conditions which cause this disease to appear in the offspring, was accepted generally and had full sway in the etiology of scrofula and of consumption of the lungs, an explanation which appears to have satisfied most medical observers as well as the

[5] Wm. H. Welch; Brit. Med. Journ. 1902, II, 1105.
[6] E. C. Rosenow; Journ. Infect. Dis. 1907, IV, 285.

public at large for a long time. With advancing facilities for the study of the disease, during its course and progress and after its termination, the theory of its direct inheritance, or at least of the inheritance of the humoral and structural abnormalities, was found insufficient, and the causes which might lead to its development had to be extended in order to account for cases in which hereditary influences in either sense had to be excluded.

Taking for a standard the normal organism with its protective functions unimpaired, any particular deficiency was properly considered in its relation to the more ready acquirement of a certain disease. The more frequently the particular deficiency preceded the appearance of the disease, the more probable appeared its causative relation. When a variety of such deviations from the normal standard had been in evidence before the outbreak of the disease, they were made the subject of separate or collective investigation, and in phthisis the number of apparently related causes became so great that Rühle [1] said truly that whatever seems capable of making man ill had been accused as a cause of pulmonary phthisis.

Of all diseases it was pulmonary phthisis which appeared to supply the most striking examples of inherited and of acquired predisposing influences, a fact which accounts for another one, namely, that in no other disease has the study of the subject been so extensive and so intensive. In the light of its bacterial origin, the theory of a direct inheritance of the disease was abandoned by most students, but that of at least an inherited predisposition to its acquirement found almost general acceptance, since the prevalence of pulmonary phthisis in families was a matter of the most common and constant observation. The numerous instances in which the disease appeared to have skipped one or more generations, and those in which no family relation could be ascertained, made it necessary to admit the possibility of its independent development and to accept an acquired predisposition. In this manner the inquiry was extended to the causes which favored and perpetuated the individual loss of the normal resistance enjoyed by the majority of the human race.

[1] H. RÜHLE; in v. Ziemssen's Handb. d. Spez. Path. u. Ther. Leipzig, V, 1874, 10.

It was but natural that the influence of other diseases, especially of those which appeared to weaken the organism and to induce a low state of nutrition, should be brought into causative relation, and that the theory to account for its acquirement should then be extended to other depressing influences, mental and environmental, including want of proper general and personal hygiene, unfavorable climatic conditions, and also physical defects as they are noted in the bodily development especially of the chest. The supposed related causes could thus be extended indefinitely and it was quite logical to include the influence exerted by food, and that of the metabolism. Finally the results of all these observations culminated in the acceptance of the dictum that anything which disturbed the physiological balance of the organism may contribute toward the advent of phthisis and is therefore a predisposing factor. We had then arrived at a much broader view of the related causes which favored the occurrence of pulmonary phthisis and, while there were investigators who considered the direct hereditary transmission, or the direct infection as the most frequent or as the only cause of the disease, others held other factors to be of equal or of greater importance.

The therapeutic measures were of course based upon the prevailing theory of cause. For the comparatively few observers who could accept only the action of direct prenatal influences, little if anything was available beyond symptomatic treatment, and the lack of accurate diagnostic methods gave them ample means for the explanation of exceptional cases which terminated in lasting improvement or in apparent recovery. More light came with the increasing evidence to the effect that phthisis was in fact an infectious disease depending upon the presence of a specific virus in the diseased organs and in their secretions and excretions. and the theory of predisposition was accordingly modified, in that this virus would prove infectious, chiefly if not solely, for those who were subject to a hereditary or to an acquired predisposition.

Our therapeutic procedures were thus broadened in scope and extent according to our expanding conceptions of the causes of the predisposition. After the discovery of the nature of the virus and of the modes by which it gains access to the tissues, the therapeutic procedures were supplemented still further by

germicidal preparations, especially of creosote which, at least for a time, became the routine prescription for the consumptive. So it came to pass that, according to the theory which the therapeutist entertained, he was an absolute pessimist, because in his conception the cause of phthisis was inherited; or if not directly so, he held that the person with a consumptive taint or stigma was doomed because he had certainly inherited the specific predisposition; if he lived long enough for an exciting cause to become operative, this would end in the disease. Or the physician was more hopeful because he expected to be able to oppose the predisposition and the progress of the disease by preventing or removing the causes as they appeared to him relevant, by adopting whatever means he believed suitable and available for the individual. In this manner our present dietetic-hygienic method of treatment was evolved, with such additions as were deemed worthy of acceptance with the increase of our knowledge.

In the meantime the increased interest in physical and other methods of diagnosis, especially the introduction of the tuberculin test, and no less the frequent autopsy-findings of tuberculous lesions, in all stages of evolution, in persons who had died from other causes and who had, at no time before, presented symptoms of phthisis, caused the more general recognition of the fact that phthisis is secondary to, and constitutes the open or destructive stage of tuberculosis, thus showing that a very large number of the supposedly predisposed class was actually in the early or purely tuberculous stage. Soon after the discovery of the tubercle bacillus, the dictum "no bacillus—no tuberculosis" found many adherents, and the contributing factors or predisposing causes, for a successful infection or for the development of tuberculosis, were more or less ignored if not entirely denied; they came to possess only a historical interest, especially for the bacteriologist to whose verdict many clinicians yielded in spite of their contrary clinical observations.

The signs and symptoms of predisposition for tuberculosis now had become those of early tuberculous disease, and those of phthisis merely indicated a more advanced stage. Prevention of tuberculosis meant the destruction of the bacillus before it gained entrance into the tissues or organs of its victims. Treatment meant to oppose the life and growth of the tubercle

bacillus and its extension in the infected organism, and naturally the earlier stages of tuberculous disease offered the best opportunity for success.

As in many other problems of the disease, actual clinical manifestations could, however, not be set aside entirely, even though the clinician had to admit, in most instances, that the signs and symptoms of a predisposition were in fact those of a preceding infection, and that, when they were present, a previous exposure could at least not be disproved. There remained, however, for the clinician the great differences in the course of the disease, which he could not ignore and which had to be accounted for. The fact that in some individuals the clinical course of tuberculosis was rapid and severe, as compared with that in others, suggested that these persons after all represented a predisposed class. The contagionists explained this difficulty by showing exciting causes for the development of phthisis, which were numerous and variable in their effect and aggravated the comparatively benign course of purely tuberculous lesions by incidental inflammations and by disturbances in the local and general nutrition of the affected part and of the body as a whole. Intervening affections like influenza, other acute diseases, trauma, exposure, and chronic affections which had previously been taken for predisposing factors, were in their view only exciting factors for phthisis, and it was asserted that these exciting causes remained inoperative without previous tuberculous disease, however slight it might be in extent. "No tubercle without the tubercle bacillus; and no phthisis or generalization without tubercle" became now the accepted dictum. The course of phthisis was apt to differ, accordingly as detrimental accidents befell or did not befall the individual. The hygienic and dietetic treatment was vindicated and, in like manner as it often prevented the advance of tuberculosis to its destructive stage, so it was believed to oppose the advance of the latter and to favor reparative processes and the arrestment of the phthisical disease.

It was quite natural that many of those whose experiences and studies had committed them to the theory of a predisposition, should continue its advocacy with whatever support they found inconsistent with its entire abandonment. In this respect they were able to offer not only valid theoretical, but also

unquestionable clinical data which, to say the least, made it appear that the subject was more complex and could not be explained satisfactorily by the simple theory of infection and tubercle-formation, and by the influence of exciting causes and detrimental influences in general, as determining the advent of destructive processes as well as their subsequent course, especially as we witness these at the bedside.

One of us has, for nearly half a century, been an interested student of this subject in all its many and variable phases, and has been able to check his own observations in the light of all the theories that existed prior to, and were advanced during this period. His own studies and those of others have, for some years, caused him to seek for additional light which might reconcile the opposing theories as they appear above briefly outlined.

Fully conscious that predisposition and resistance are in reality relative quantities, it appeared to him probable that the resistance might be specific and that, even if it were not transmissible from parent to offspring, there was at least no valid objection to the assumption that it might be acquired in the course of the disease. The study of Reibmayr's publication, which was referred to in the preceding chapter, was especially conducive to considerations of this kind and, as will appear in the practical and experimental details in Parts II and III, he has met with much encouragement and support in his belief that both contingencies are possible and that, if these studies are continued, they will in time tend to reconcile the opposing views in regard to predisposition.

The demonstration, in the latter part of the last century, that certain microörganisms stand in causal relation to certain infectious diseases, raised questions without number, to which answers were urgently required, in order to explain the interaction between the invading bacteria and the invaded host. It needed to be explained not only how certain bacteria can cause certain diseases, but also why their invasion is not always followed by the related disease; for it had soon been found that not every infection leads to disease but that, under certain conditions, the organism is capable of resisting the pathogenic action of the bacteria. The interaction between bacteria and

organism thus became a question of the relation between action and reaction, or between infection and resistance, and it was necessary to study the mechanism of this resistance.

The subject of resistance to infection and to infectious disease fills some of the most interesting and fascinating chapters of the records of medical research. The study of the nature of resistance, its cause or causes, the modes of its manifestations, and the causes of its failure has given rise to various theories designed for its elucidation. It is not our purpose to critically examine, or even to relate in detail, the several theories now in vogue, which attempt to account for the various phenomena of natural resistance to the acquirement of certain infectious diseases, as they are observed in families and species of animals including man. The theory of adaptation, selection, and survival of the fittest, with the ultimate hereditary transmission of acquired or favorably modified defensive functions or protective provisions, is perhaps the most generally accepted and, if carried far enough, it can be made to include all other theories and to apply to the natural immunity of species, races and individuals.

The Soil Theory explains the relation of natural resistance to certain bacterial diseases by conditions of metabolism, of temperature, etc., because of which the soil is chemically or physically favorable or unfavorable for the propagation of a particular kind of invading bacteria. The importance of metabolic conditions is supported, for instance, by the fact that dogs are naturally immune to infection with anthrax, although their blood serum is not capable of destroying anthrax bacilli *in vitro;* also by the observation that the diet seems to have an influence upon the occurrence of bacterial diseases, since these are often different in carnivorous and in herbivorous animals. Bacteria may, however, become adapted, by repeated passage, to the conditions of metabolism and of temperature in an organism for which they were originally not virulent, and may in this manner become pathogenic for the species, the natural resistance of which is then suspended for the particular strain of the bacterium.

The importance of the metabolism for the creation of a favorable or unfavorable soil is shown by the great sensitiveness which many bacteria show to changes in the composition of

their artificial culture media, and to variations in the temperature at which they are grown. It has further been asserted that it is manifest in the influence of the blood-alkalinity upon the propagation of bacteria in the organism, since, in its variations, this is likewise an expression of different metabolic processes.

THE BIOCHEMICAL THEORY is of more recent date and is the outcome of modern research in the problems of immunity. It postulates the necessity of a suitable receptor-apparatus, on the part of the cells of the host, identical with or fitting to corresponding "receptors" of the bacterial cells or poisons, which then can unite with and become anchored to the cells of the host. If the cells of the host do not have fitting receptors for the bacterial cell or its toxin, no union can occur; the cells of the host are not injured, and the bacterial cell or toxin is consequently not pathogenic for them and cannot produce disease.

Whatever theory may be adopted for the interpretation of the facts which have accumulated through observations of past centuries, the facts themselves are indisputable; they are subject to daily confirmation and may be summarized in the following manner:

(1) Certain infectious diseases do not occur spontaneously in certain species or races of animals, even after marked degrees of exposure to infection, whereas the same infectious diseases do occur spontaneously in other species.

(2) In the former, artificial infections with the respective microörganisms are resisted likewise, whereas like degrees of artificial infections cause the latter to acquire the related diseases and to succumb to them under certain conditions.

(3) In order to overcome the resistance of the former, it is necessary to resort to large doses of the virus, or to special modes of infection, or to the adoption of special measures which are calculated to interfere with, or to damage or impair the normal functions of their organs.

(4) When the resistance of the former, to an ordinary mode of infection, appears to be incomplete, the infection is followed, as a rule, only by a local lesion, whereas in the latter the disease is not so restricted and follows its normal course.

CELLULAR AND HUMORAL RESISTANCE

In the further study of resistance to infection, the exhaustion theory of Pasteur, the retention theory of Chauveau, the phagocytic theory of Metchnikoff, the humoral theory of Buchner, were advanced among a number of others. Several of these theories proved inefficient and have been abandoned; but the two most important ones, namely, the phagocytic theory and the humoral theory, have survived and form to-day a basis for the explanation of most observed facts. While at first they stood in sharp opposition one to the other, the advancing and expanding knowledge of recent years has led to the conviction that both are required to explain satisfactorily what each one had attempted to elucidate alone.

It may be accepted as a general proposition that the theory of cellular resistance, especially as formulated in the phagocytic theory, serves particularly to account for the natural, generic and species resistance or immunity to infection; but it becomes insufficient and requires the support of the humoral theory as soon as it is a question of an acquired, or of an artificially induced immunity to an infection to which the organism is naturally more or less susceptible.

The Cellular Theory of Metchnikoff

The phagocytic theory of Metchnikoff, as cited by Levaditi [8] is, in brief, as follows: Whenever an organism, which possesses a natural (congenital) or an acquired immunity to the pathogenic action of a certain virus, is invaded by that virus, a struggle takes place between it and the phagocytes of the invaded organism. In this struggle the phagocytes are victorious in so far as they take up the microbes within their protoplasma-bodies, digest them more or less and thus render them harmless. Metchnikoff sees in phagocytosis not a simple mechanical incorporation, but he holds that the ingested bacteria are injured and, in the most favorable event, digested and destroyed within the leucocytes, and that the immunity to their invasion or to their pathogenic power is manifested in this manner.

[8] C. Levaditi; in Kraus & Levaditi, Handb. d. Technik u. Methodik d. Immunitätsforschung; Jena, II, 1909, S. 279.

Older observations of Klebs, Waldeyer, Birch-Hirschfeld and others have shown that bacteria are, in the living body, taken up by wandering cells. It has also been shown experimentally, by Birch-Hirschfeld, Wyssokowitsch, and others, that saprophytes introduced into the circulation are, like other foreign corpuscular elements, taken up by leucocytes and removed from the circulation. Panum referred, in 1874, to an observation of Ravitsch that, if bacteria are injected into the blood of living animals, they disappear rapidly and perish. As early as 1881, K. Roser pointed out the relation between resistance to infection and phagocytosis, when he said that immunity to infection depends, in his opinion, upon (1) the relative salt-content of the tissue fluids, and (2) the ability of the contractile cells of the organism to take up the invading germs.

It is of interest that Koch [9] mentioned the intracellular position of the tubercle bacilli, in his first communication on the etiology of tuberculosis, where he refers to the fact that the bacilli are often seen enclosed in cells, although many are free. If giant cells are present, the tubercle bacilli are found preferably within these cells. In his second communication [10] he considers the rôle of the wandering cells in regard to the dissemination of tubercle bacilli, for which these cells are of importance. He states that, taking up a bacillus, a wandering cell can not go further after it has lost its motility under the influence of the parasite. At the place where it is arrested a new tubercle-nodule must develop. In this manner, secondary foci at distant places, originating through metastasis from the first tubercle-eruption, are readily explained. Koch does not refer to any injury which the tubercle bacilli suffer through the action of the leucocytes, although he finds that they may die out in the giant cells; usually, however, the cells themselves appear to suffer through the action of their bacterial content.

According to Metchnikoff [11] the study of these conditions is particularly difficult in tuberculosis, because, in the manipulation of specimens (staining, heating, etc.), many of the cells loaded with bacilli burst and discharge their contents. Metchnikoff expressed the opinion that the leucocytes must accustom

[9] R. Koch; Berl. klin. Woch. 1882, XIX, 221.
[10] R. Koch; Mittheil. a. d. kaiserl. Gesundh'-amte. 1884, II, 1.
[11] E. Metchnikoff; Virchow's Archiv. 1884, XCVII, 502.

themselves gradually to the ingestion of substances which they avoid ordinarily, and that they acquire the ability of ingesting virulent bacteria by first taking up attenuated ones. He thinks it possible that the giant cells injure tubercle bacilli by secreting an acid substance which is responsible for the destruction of the intracellular bacteria. It is also possible that after a phagocytic cell has injured an ingested bacterium, it dies and is, together with its content, taken up by another cell which then completes the destruction of the bacterium.

The phagocytic theory was not the result of deliberate studies undertaken to ascertain the reaction of the organism to infection; at least it was not so originally. The primary studies of Metchnikoff were undertaken with a view of clearing up the question of intracellular digestion and absorption of foreign elements in the interior of invertebrates, which had been denied. When the actual occurrence of such an intracellular digestion had been demonstrated, the question suggested itself whether the same could also be shown to occur in vertebrates. As an application of the results of his related studies, Metchnikoff then declared at the Naturalists' Congress in Odessa, in 1883, that in his opinion the cause of recovery from infection is found in the ingestion of the virus within certain leucocytes of the infected organism, which he called *phagocytes* ("eating cells"), and in its intraprotoplasmatic digestion and destruction. In the article which appeared the next year in *Virchow's Archiv* he expressed the view that phagocytosis occurs as an active defense, as an expression of immunity, since he observed it, for instance, in animals which are immune to anthrax, but not in susceptible animals. This declaration of Metchnikoff started a long series of studies, undertaken by himself and his pupils, for the purpose of substantiating and confirming his hypothesis, and of meeting the numerous objections which did not fail to be voiced, especially by German investigators. In course of time the practically exclusive rôle of defense against infection, which Metchnikoff attributed to the phagocytes, was modified in order to account for certain phenomena indicative of immunity, in which no cellular activity could be shown, and the phagocytic hypothesis was extended to suit the requirements of observed facts and to meet the opposing humoral hypothesis. This had become necessary especially when

it had been shown that leucocytes, which are entirely freed from serum, are almost impotent against bacteria.

According to these modifications, antibacterial substances, the presence of which in the tissue fluids is postulated by the humoral theory, are acknowledged by Metchnikoff, who calls them *stimulins;* but he maintains that they are formed only in consequence of a preceding *phagolysis* or disintegration of phagocytes, through which these substances are liberated and taken up in the serum. Further, he claims that the bacteriotropic substances, opsonins, etc., which are supposed to prepare bacteria for phagocytosis, are also derived from leucocytes.

The cells which appear to have the function of phagocytes, are what Metchnikoff calls the *microphagi,* that is, polynuclear leucocytes and small lymphocytes, and the *macrophagi,* which are principally the large mononuclear leucocytes, the ameboid cells of spleen and lymphatic glands, the alveolar cells of the lungs, and certain fixed cells, namely, endothelial cells of serous membranes and lymph spaces, bone corpuscles, giant cells of bone-marrow, and so on. Metchnikoff believed the wandering cell to be of far greater importance for immunity, that is, for the defense of the organism, than are the fixed phagocytes; they accumulate in immense numbers at the point of an injury or infection and take part in the inflammatory reaction. The leucocytosis occurring in the course of infectious diseases is due to an increase of these wandering cells, and they probably furnish the substances which determine the activity of the immune serum.

In the immune organism, the phagocytes are attracted to the invading bacteria, as well as to other foreign corpuscular elements, red cells, etc., by a force which has been called *positive chemotaxis.* It has been observed that highly virulent bacteria may repel the cells, by *negative chemotaxis,* and this has been explained also in such a manner that the approaching cells are injured by bacterial poisons (e. g., aggressins) and thereby become unable to ingest the bacteria. The ingested bacteria may continue to grow in the interior of the phagocytes and eventually destroy them, and in tuberculosis this probably takes place in the non-immune organism. Phagocytosis is therefore not necessarily identical with disintegration and destruction of the bacteria and may even be a factor in their dissemination.

Since, under ordinary conditions, living protoplasm cannot be digested, the question is pertinent how living, virulent bacteria are injured or prepared for intracellular digestion. Metchnikoff affirms that the phagocytes secrete an acid substance which is inimical to bacterial life. In explaining the observation that blood, freed from all cellular elements, is capable of killing bacteria, he asserts that the antibacterial substances, derived from disintegrated leucocytes, are of the nature of ferments; he calls them *cytases.*

Pettersson [12] of Stockholm, who has studied the question very carefully (for literature, see the review of Kling [13]) found that the leucocytes of animals which are immune to anthrax, and to a lesser degree also those of susceptible animals, contain bactericidal substances which differ from the bactericidal serum-substances, particularly in their greater resistance to heat. They are liberated only by the destruction of the cells, and this author designates them as *endolysins,* in analogy to endotoxins and endo-enzymes. According to Weil [14], endolysins are not specific as are the serum-substances, and this is in support of the view that phagocytosis is a function of natural immunity or resistance. It is believed probable that the endolysins act upon bacteria which have been phagocytosed, thus causing their intracellular destruction, and that they act also upon free bacteria after having been liberated through the disintegration of the leucocytes. According to this view, the endolysins would closely resemble the normal opsonins or bacteriolysins, and this would support the contention of Metchnikoff that these important substances, contained in the body-fluids, are derived principally from the cells which he classes as phagocytes.

It seems to be a fact that the first reaction of the animal body against infection, for instance with the tubercle bacillus, is manifested by an accumulation of polynuclear leucocytes, and most authors agree that the white blood-cells must be accepted as provisions of the defense of the organism against the tubercle bacillus. In this defense some authors attach the greatest importance to the polynuclear leucocytes, others to the large mononuclear cells, still others to the lymphocytes. Kling's

[12] ALFR. PETTERSSON; Zt. f. klin. Med. 1907, LXIII, 79.
[13] C. A. KLING; Zt. f. Immunitätaforsch. Orig. 1910, VII, 1.
[14] E. WEIL; Arch. f. Hyg. 1911, LXXIV, 289.

experiments seem to prove that, in relatively resistant animals, the polynuclear leucocytes are able *intra vitam* to destroy human tubercle bacilli or to attenuate their virulence, and he leaves open the question of the relative importance of the large mononuclears and of the lymphocytes, which, according to Schneider, do not seem to contain as much endolysin and are not as active in the phagocytosis of virulent bacteria as the polynuclear leucocytes.

The Function of the Leucocytes

In consideration of the importance of cellular resistance, it appears proper to make some special reference to the function of the leucocytes. Their real purpose seems to be to take up foreign corpuscular elements and to remove them from the tissues. This function is seemingly not selective, but affects living as well as dead bacteria, and not only these, but all sorts of other corpuscular substances, such as alien red blood-cells, India ink, coal-dust and soot, and so forth. It is manifest that this ability of the leucocytes, to take up corpuscular elements may produce various conditions, according to the kind and nature of the ingested substances and according to the ability of the cells to deal with them.

(1) If the leucocytes ingest living, virulent bacteria, their nonselective phagocytosis is productive of harm, either to the organism or to the leucocytes themselves. Not being capable of dissolving and digesting the bacteria, the leucocytes may perish, they may carry the bacteria to other parts of the body, or may even protect them against the bactericidal tissue fluids.

(2) If the corpuscular elements are not living, the question will arise whether they are soluble or not. Insoluble substances, such as particles of coal-dust, simply are removed from the circulation and deposited elsewhere.

(3) On the other hand, dead microbes may be subjected, within the leucocytes, to the action of their digestive ferments and are then dissolved more or less completely, just like other more or less soluble corpuscular elements which are not injurious to the organism in themselves, and are eliminated from the circulation in this manner. It is here that an essential advantage probably accrues from the action of the leucocytes;

but this does not in any way stand in relation to the destruction of the bacteria.

It may be said, therefore, that phagocytosis is the ability of the leucocytes to ingest heterologous substances and to digest them. The digestion does not run parallel with the ingestion, but takes place in only a small number of cases. It need hardly be pointed out that the function of phagocytosis must not be confounded with the action of the leucocytary bactericidins, because these are soluble substances which are secreted by the leucocytes and are discharged into the circulation.

The Humoral Theory of Buchner

Metchnikoff's theory of immunity was strongly opposed by various authors, for instance, by Nuttall [15] and others of Flügge's school, who showed that blood-plasma and serous exudates were capable of destroying bacteria *in vitro;* and principally by Buchner [16] who confirmed Nuttall's findings, and demonstrated the bactericidal action, *in vitro,* of blood-serum that was freed from all corpuscular elements. Buchner referred this action to certain protective substances in the serum itself, which he called *alexins* (from *aleixein,* to protect), and he suggested, even before Metchnikoff, that his alexins might possibly be derived from the leucocytes.

Buchner also found that the bactericidal power of the blood-serum is destroyed by heating to 56°C. for ten minutes or more, that exposure to sunlight and standing at ordinary room temperature lowers it. He looked upon the protective substances in the serum as being preexisting in the blood, and attributed to them the ultimate cause of immunity.

It was thus claimed, against Metchnikoff, that phagocytosis of bacteria takes place only after the latter had been killed, or at least weakened, by these antibacterial substances of the blood; that therefore phagocytosis was more a function of scavengers than an actively bactericidal action. In support of this view it was affirmed also that phagocytosis goes by default in case of highly virulent infections where there is the greatest danger, and further that in immune animals many bacteria are

[15] G. NUTTALL; Zeitschr. f. Hyg., etc. 1888, IV, 353.

[16] H. BUCHNER; Arch. f. Hyg.; 1890, X, 84, 101, 121, 149, and elsewhere.

destroyed without being enclosed in cells at all. The unqualified assertion was advanced that the struggle against invading bacteria depends primarily upon the germicidal action of the tissue-fluids, and that phagocytosis is not a bactericidal process at all, but that it merely serves the purpose of removing the killed bacteria. This was the teaching of the humoral theory.

A mediation between the two opposing theories was attempted early by Lubarsch [17] who pointed out the contradictions contained in both, and their inability to serve, each one by itself, as an explanation of immunity. Rejecting the suggestion that he was an "unconditional adherent of Metchnikoff," he concluded, from a careful examination of the evidence, including that of numerous experiments of his own, that phagocytosis is not an absolute protective provision in the struggle of the animal organism against bacterial invasion, and that its importance is subordinate. He was convinced that extracellular antibacterial influences are of moment, although he refused to admit their exclusive importance entirely independent of cellular activity. Lubarsch held that it was too early to attempt a definite theory of immunity and that much more material must be collected before this would be possible.

During the following years many valuable and pregnant discoveries contributed to secure a rapprochement of the two opposing theories with the result that, at present, both are drawn upon to explain the phenomena of immunity. One of the earliest of these discoveries was that of bacterial toxins and of the fact that their parenteral [18] introduction gives rise to the formation of antitoxins which neutralize the action of the toxins quantity for quantity; another was the discovery of what is known as PFEIFFER'S PHENOMENON which proved the interrelation of humoral and cellular protective forces; further the demonstration that the organism responds to the parenteral introduction of foreign red blood-cells in a similar manner as it does to that of bacterial cells or of any other foreign protein.

[17] O. LUBARSCH; C. f. Bakt. 1889, VI, 481.

[18] The term *parenteral* has been introduced only recently and is not found in many medical dictionaries. It is derived from the Greek—*para*, beside, and *enteron*, intestine—and is used to designate the introduction of substances into the organism through other avenues than that of the digestive tract, such as subcutaneous, percutaneous, intravenous, and so on.

It is especially through the last-mentioned discovery, that of HEMOLYSIS, that the study of this response was greatly facilitated and the discovery of the bactericidal and bacteriolytic substances, as well as the theory of their mode of formation and of action was made possible. Of particular influence in the adjustment of the differences between the cellular and the humoral theory, however, was the discovery of bacteriotropic substances or opsonins.

Making use of the most serviceable factors of the cellular and the humoral theory, Much [19] recently attempted to formulate a survey of the mechanism of organic resistance to bacterial infection. Inquiring into the means which the organism has available for the destruction of invading bacteria and for the elimination of their toxic products, he finds that the body normally possesses disinfecting bactericidal agencies, that is to say, substances in solution which can be demonstrated in the tissue-fluids and are designated as normal bactericidins. There are two varieties of these substances:

(1) Humoral bactericidins (serum-substances),

(2) Leucocytary bactericidins (plasma-substances).

The exact origin of the humoral bactericidins is not known; the leucocytary bactericidins originate in the leucocytes and are possibly identical with fibrinogen. They occur only in plasma, that is, in the uncoagulated, not defibrinated blood which has been freed of its cellular elements. The serum-substances occur in both, plasma and serum. Serum and plasma are not identical in their action upon bacteria, some of these being destroyed only by one, others only by the other, while still others are injured by both.

The action of the leucocytary bactericidins is a simple one; that of the humoral bactericidins depends upon the cooperation of two components one of which is thermolabile (complement), while the other is thermostable (amboceptor). The thermolabile component of the humoral bactericidins, in heated or inactivated serum, can be restored by the addition of fresh serum (complement); but this is not possible in the case of the leucocytary bactericidins, once the serum has become inactive. It follows that the humoral bactericidins require com-

[19] HANS MUCH; Die Immunitätswissenschaft. Würzburg, 1911, 71.

plement for their action, and the leucocytary ones act without it, which, by the way, would contradict the opinion that the complement is derived from the leucocytes.

Both bactericidal substances appear to be non-specific in that they are active against all bacterial infections. When, however, it becomes necessary, owing to massive or virulent infection, to mobilize unusual amounts of protective forces, the increase concerns mainly the humoral bactericidins which are then always specific.

According to Much, therefore, the struggle against infection is carried on in such a manner that the humoral and leucocytary bactericidins are mainly responsible for the destruction of the invading bacteria, and that the task of neutralizing and of eliminating the endotoxins, liberated after the destruction and disintegration of the virus, falls to the leucocytes. It is assumed that phagocytosis affects only bacteria which have been killed or weakened, and that phagocytes cannot digest virulent bacteria even if they ingest them. This is illustrated and supported by the fact that phagocytosed gonococci certainly are not killed or rendered non-infectious in the process of phagocytosis, and that many other bacteria (lepra bacillus, tubercle bacillus, etc.) seem to be transported from place to place by the leucocytes, without being always or necessarily injured in their pathogenic power.

The actual struggle against infectious microörganisms appears thus to be carried on conjointly by both the humoral and the cellular forces, both being equally necessary. While the bactericidal substances exert their destructive action upon the bacteria, the leucocytes prevent them from discharging their endotoxins into the circulation, and render them harmless; they assume the rôle of crematories and are, themselves, ultimately destroyed in the fulfilment of their function.

CHAPTER III

ACQUIRED SPECIFIC IMMUNITY

The observation that some persons resist, or at least overcome, an attack of an epidemic disease, is old, and so is also the further observation that persons who previously had recovered from certain diseases of this kind, are usually not affected again during subsequent epidemics. While, in the minds of the ancients, these occurrences were generally attributed to a special favor of the deity, it is said that the Chinese applied them deliberately, as early as the eleventh century, to the production of immunity against smallpox, by inducing a mild attack of the disease, which was done by introducing the infectious poxcrusts into the nostrils. In Persia the powdered crusts were introduced by friction into small incisions in the skin, and other Oriental peoples had similar customs designed to accomplish the same purpose. This method of producing an immunity artificially, by causing the related disease in a mild form, was introduced in Europe, in 1721, by Lady Montague, the wife of the British Ambassador to the Porte, who had practised the so-called Greek method of inoculation against smallpox on her own children. It was observed that, introduced in this manner, the disease usually took a milder course than after its ordinary epidemiological acquirement, while the protection resulting from it appeared to be equally efficient. This observation led to the application of the method for the prevention of other diseases, healthy children being put to bed with those ill with measles, and infants being exposed deliberately to whooping cough and to other infections. Inoculation was practised for the prevention of measles, scarlet fever, bubonic plague, cholera, and also of epidemic diseases of animals, like infectious pneumonia and rinderpest. Although this procedure afforded an opportunity of accumulating much experience, the outcome did not always result favorably to the method of protective inoculation, because the induced attacks often were severe, and also because

not infrequently they gave rise to epidemics of the related dis ease.

It had long been known in many parts of Europe that persons who were infected or inoculated with cowpox, were thereby protected against smallpox. This knowledge was first made use of deliberately by Edward Jenner, an English country physician who, on May 14, 1796, crowned his investigations extending over many years, by vaccinating, and thereby actually protecting a boy, James Phipps, against smallpox, by means of producing an attack of cowpox or vaccinia. The vaccine was taken from a pustule on the hand of a dairy maid, Sarah Nelmes, and was therefore what is to-day called "humanized lymph." Jenner proved the success and efficiency of the vaccination by inoculating the boy, some time later, with smallpox which the latter resisted. The first account of these studies was published by Jenner in 1798.[1]

The correctness of Jenner's method was soon acknowledged, although not without much opposition; with the increasing practise of vaccination, the distribution of smallpox in Europe and in our country became noticeably less. In course of time the conviction became general that the virus of cowpox was in fact an attenuated smallpox virus, but no advantage could be taken of Jenner's discovery, nor could its underlying principle be applied to the prevention of other diseases in the state of knowledge at that time, as the modern researches in immunity and immunization were inaugurated only in the seventies of the last century by Pasteur and by Koch.

For a long time smallpox remained the only disease in which the artificial production of a specific immunity appeared practical. No safe vaccines could be found for other infectious diseases, their actual viruses being unknown. Moreover some diseases apparently failed to confer a subsequent protection even after repeated attacks, and only speculative theories could be advanced to account for the resistance to a particular disease, which was acquired in this manner, while it failed in the case of other diseases. When, however, the relation of bacteria to certain infectious diseases received increasing consideration in the second half of the last century, the studies in bacteriology

[1] Edward Jenner; Inquiry into the Causes and Effects of Variolæ Vaccinæ. London, 1798. German translation, Leipzig, 1911.

made rapid strides under the guidance of Pasteur, of Koch and others, and brought light into the obscure domain of the etiology of infectious diseases, as well as of the immunity against them. It then became possible to study and determine experimentally the manner in which an acquired immunity develops in response to an attack of an infectious disease which is recovered from, and inferences could be drawn for the artificial production of a like immunity, for the purpose of preventing disease, or of treating it with better success in patients in whom the natural defensive forces are not sufficient to develop it and thereby to overcome the disease.

After the early difficulties in the isolation and culturing of the related virus had been greatly reduced, in the case of several infectious diseases in which the specific causative microorganisms had been discovered, data were secured step by step, which made possible the experimental study of some of the infections. In so far as a particular microörganism had been proved as the true etiological agent of the disease, it became possible to experiment with it, with a view of obtaining this virus in a modified and less dangerous form by means of which protection might be produced against a serious and frequently fatal attack of the disease, by inducing a mild attack, even as Jenner had done many years before. Thus the study of bacteriology not only afforded light in etiology, but made possible the experimental studies in specific immunity which develops in consequence of an attack of a given infectious disease, even though its mechanism still remained obscure. Bacteriology also advanced and perfected our means of recognizing and differentiating infectious diseases, that is, their diagnosis.

BACTERIAL TOXINS

In these bacteriological investigations and experiments, it was found that the manifestations of an infection caused by bacteria, which have gained entrance to the tissues, may be twofold; they may be chiefly those of a toxic action or those of structural alterations of tissue. As a rule, both are associated, but the principal danger to the organism arises in some diseases from toxins which are absorbed, as in diphtheria or tetanus, and in

which the structural alterations produced at the point of localization of the bacteria are comparatively slight; indeed none at all may be observed in tetanus; while in other diseases it is the tissue-reactions in the shape of exudative inflammation, as in pneumonia, or tissue-necrosis as in tuberculosis or syphilis, which are concerned chiefly in threatening the life of the patient.

Toxins which are produced by bacteria in their living state, by active secretion or excretion, are commonly spoken of as *exotoxins*, in contradistinction to other toxic substances which are constituents of the bacterial bodies, and are referred to as *endotoxins*.

Although the secretions and excretions of other bacteria, for instance, of the tubercle bacillus, also have a toxic action, and their absorption or parenteral introduction likewise gives rise to specific antibodies which can bind and neutralize them (but only with the aid of a ferment contained in the blood-serum), the exotoxins of a small group differ from them in that they are highly poisonous to the animal organism without its previous sensitization, that they threaten the life of the infected organism directly, that they appear to be of the nature of a ferment, that their absorption or artificial parenteral administration causes the formation of antitoxins which neutralize their related toxins quantity for quantity without the cooperation of another ferment. The bacteria which secrete such exotoxins are designated as *toxin-bacteria*, and their toxins as *true bacterial toxins*. True toxins can be obtained from the filtrates of cultures of the respective bacteria grown upon liquid media, and the filtrates are used for the direct or *active immunization* of animals, as a rule, horses, in whose sera the antitoxin appears in response to the treatment.

True bacterial toxins were demonstrated first for the diphtheria bacillus by Roux & Yersin in 1889, and for the tetanus bacillus by Kitasato in 1891. It was then expected that analogous poisons could be found and isolated from cultures of all pathogenic bacteria, but the list of true toxin-producers has remained small; it includes the Bacillus botulinus, the Bacillus pyocyaneus, the Bacillus of dysentery and that of symptomatic anthrax. Other true toxins of vegetable and animal origin were discovered, however; among others, ricin, abrin, ro-

bin, snake venom and other reptile poisons, the study of which materially promoted that of specific antitoxic immunity.

The diseases of the human subject, which are produced by toxin-bacteria and in which the true toxins endanger the life of the patients, are more or less acute, and their treatment with antitoxic sera is therefore not required for prolonged periods of time.

Other bacteria, whose pathogenic action is manifested chiefly by degeneration and by other marked destructive changes of the tissues which they invade, produce no true toxins in the sense described above. The damage to the tissues, which follows their growth and multiplication, is believed to result from another form of toxins, of the nature of proteins or of lipoids, which are supposed to be body-constituents of the bacteria and to enter into the surrounding media only after their death and disintegration. These toxic substances therefore were called *endotoxins* by Pfeiffer, who was the first to study them and believed that they are pre-formed within the bacterial cell-bodies. Like the true toxins, they are specific for each bacterium. Their absorption or parenteral introduction causes the production of bactericidal or bacteriolytic reaction-products which may appear also in the sera of individuals affected with the particular diseases, and after recovery from them. Spontaneously they are produced, as a rule, in but small quantities, but after artificial immunization their demonstration is often possible in what may be considered as large amounts. These substances influence the virulence of the related bacteria adversely or destroy it entirely, and they may likewise alter them morphologically in varying degrees or even cause their complete disintegration. These morphological changes are called *bacteriolysis,* or, as they affect red cells, *hemolysis,* or then by the general term of *cytolysis* (from *kytos,* a hollow space, a cell); the antibodies, which are responsible for the process, are known as *bacteriolysins, hemolysins* or *cytolysins,* and the immunity to the bacteria, which is produced through the formation of bacteriolysins, is spoken of in general as *antibacterial,* or in particular as *bactericidal* or *bacteriolytic.*

The discovery that the parenteral introduction of the bacteria themselves, or of their products of secretion or excretion, gives rise to the formation of reaction-substances or *antibodies,* and that organisms treated in this manner may become resistant to

the pathogenic action of the related bacteria or bacterial products, revolutionized profoundly both the theory and the practise of medicine and led medical endeavor into entirely new channels.

In seeking an understanding of the mechanism of immunity, it was natural to bring the observed resistance to a second attack of the same disease into relation with the humors of the living body, notably the blood which was then first studied seriously in health and in disease and also after recovery from disease. Metchnikoff's observations and those of Buchner, to which we have referred, form the basis of all subsequent theories intended to explain the mechanism of natural and of specific resistance or immunity.

The related serological studies led to the discovery of several biological and biochemical reactions which are peculiar to sera obtained from animals and human subjects affected with certain infectious diseases, and from individuals who have been treated or immunized with certain pathogenic bacteria or with their products. These reactions supplied the student with a guide in his further investigations and, although the interpretation of their significance and import varies, they have nevertheless advanced our knowledge of antibacterial immunity in a degree which we cannot conceive to have been possible without their aid.

The reactions referred to are based upon the fact that the biochemical character and structure of the several types of cells and of organized albumins in a given species of animal is peculiar to that species; consequently like substances derived from other animals, from plants or bacteria, are foreign to the animal organism, although primarily they are built up of like molecules and atoms; being specific for each species, they cannot serve for a direct substitution. In order to utilize foreign substances in the building up and in the replacement of its own tissues, the organism is obliged to change their molecular and atomic structure by dissociating and then reconstructing them in such a manner that they correspond with its own. As the foreign substances are of highly complex organization, their reduction must be carried far enough for the foreign character to disappear, and then the organism is enabled to utilize them. This reduction proceeds from the complex and chemically less stable to

the simple and chemically more stable atomic state, while the reconstruction takes the reverse course. These reduction-processes occur normally in the course of gastro-intestinal digestion, and the reconstruction may be said to constitute the process of assimilation. Combined, these functions stand in relation to the growth and replacement of the tissues.

In case such foreign substances are introduced through other avenues than the digestive tract, its functions are not available for their reduction, and if the organism did not have at its disposal an auxiliary function for such an emergency, these substances would continue in their foreign state and, according to their nature, they would either poison or otherwise threaten the integrity of the tissues and even life itself; or they could become oxidized or be eliminated or disposed of in other ways without detriment to the body. This auxiliary function, therefore, appears to be a provision of nature for the protection of the organism in time of need. Apparently latent until required, it becomes manifested on being stimulated by the first introduction of the foreign substance. It appears to be strictly specific in that one foreign substance does not cause it to be activated for all others; on the contrary, it becomes active only for substances which have an identical biochemical constitution and for no others. By this provision the organism is enabled to reduce a particular foreign substance in a like or similar manner as occurs in gastro-intestinal digestion, and it can make use of the resulting elementary products in the building up and replacing of its own tissues by selection and rearrangement as may be required.

The presence or absence of this function in its active state, for any particular foreign cell or protein, is subject to demonstration by biological tests which are made by subjecting the cell or protein to the action of the blood-serum of the individual. In case the function is found to be absent on a first test, its subsequent development can be shown by repeating the test after the introduction of the foreign cell or substance parenterally, i.e., by other avenues than the digestive tract. The rapidity with which the function develops to such a degree that it may be subject to demonstration, varies with the nature of the foreign substance, with its solubility and with the amount which has been introduced; it can be increased to a certain extent by repeated introduction. The function itself is supposed to depend upon the for-

mation of specific substances which are produced by the tissue-cells in response to their stimulation with the particular foreign substance. In the nomenclature used for describing the reactions, these specific substances are designated generally as *antibodies* or *immune substances,* and the foreign substances which stand in relation to their formation are spoken of as *antigens.*

The simplest example of specific antibodies is that of antitoxins which neutralize the related toxins, but do not act against other toxins or against their related bacteria. Other antibodies, which have antibacterial rather than antitoxic properties, agglutinate or precipitate their antigens, or they dissolve them and, in the case of bacteria, they destroy their virulence and viability. Still others may influence their antigens in such a manner as to render them susceptible to the phagocytic action of the body cells.[2]

The observation of antibodies against specific bacteria, in the serum of normal individuals who give no history of having previously experienced an infection with them, or who have not been immunized with these specific bacteria or their constituents, is mentioned by all authors who consider their relation to acquired immunity. It has been claimed that certain kinds of antibodies may exist as "natural" or "normal" antibodies; for instance, agglutinins and opsonins for the tubercle bacillus have been demonstrated in the blood-serum of non-tuberculous persons.

Ehrlich explains the occurrence of normal antibodies upon the ground of his side-chain theory, by a stimulation occurring under the physiological hyperactivity of the cells in their nutritive and metabolic functions, whereby cell-receptors are likely to be produced in excess and some, although in smaller number than under the stimulation of a specific infection, are then cast off and appear free in the serum. Their presence in immune serum, in larger amounts than observed in normal serum, is therefore supposed to be necessary before it would be justified to accept the positive reaction to stand in relation to specific stimulation with bacteria or their products. We have hereto-

[2] Some authors insist upon the unity of the several antibodies. According to them, the various immunity-reactions simply represent different manifestations of the same antibody under different conditions.

fore sought light on the presence of "normal" antibodies against the tubercle bacillus as regards their passive transmission from the mother to the offspring, and have referred to our observations in the first chapter. It may also be reasoned that normal antibodies, which are demonstrated in the blood-serum of apparently healthy persons, have originated in response to an infection which was too slight to cause any symptoms whatever and, applying these various possibilities from the individual to the race, that their development has become a racial characteristic through centuries of contact with the related disease.

It must be taken into consideration, however, that disease is not necessary for the development of antibodies, but that they may be produced in response to an infection which is not followed by it; indeed, it may be claimed that disease may be prevented by the very fact that the reaction-products are elaborated in sufficient quantity and quality so as to inhibit the multiplication and the pathogenic action of the related bacteria; in short, so as to prevent disease.

The most important one of the reactions under consideration is undoubtedly that of complement-fixation, the discovery of which was, however, preceded by that of several others, for instance, the agglutination of certain bacteria when they are brought in contact with sera from subjects who had been infected or treated with these bacteria or with their products. Not all of these reactions can be said to be immunity-reactions in the sense that they indicate a distinct resistance to the related bacteria; some of them appear to have only diagnostic significance and the antibodies which they indicate do not, by themselves, exert any bactericidal function.

AGGLUTINATION

Agglutination is the power of the blood-serum of an animal or a person, infected by a certain bacterium, to clump or agglutinate the bacteria when it is added to them in a test-tube. Agglutination is active against formed proteins, and it is closely related to the reaction of precipitation which is active against proteins in solution.

The reaction of agglutination of typhoid bacilli, which was

the first practical adaptation of the method and has remained the most important one, is described by Kolmer[3] as follows:

"*If typhoid immune serum from an immunized animal or from a patient suffering from typhoid fever is added to an emulsion of typhoid bacilli in a test-tube, and the mixture is placed in an incubator, the following phenomenon will be observed: the bacteria, which previously formed a uniform emulsion, clump together in little masses, settle at the sides of the test tube, and gradually fall to the bottom, the fluid becoming almost clear. In a control test in which no active serum is added, the fluid remains uniformly cloudy. If the reaction is observed microscopically in the hanging drop, it is noted that, with the addition of the serum, the bacilli move nearer and nearer one another, this process being followed by a gradual loss in motility and the formation of clumps. The substance in the serum causing this phenomenon is called agglutinin.*"

Although it had been observed before by Metchnikoff, Issaeff, Washbourne, Bordet and others, agglutination of bacteria by their related immune sera was first described in 1896 by Gruber & Durham,[4] to whom the recognition of its importance must be credited. Soon after, Widal[5] showed that agglutination in typhoid fever permits a diagnosis to be made during the course of the disease, and thus this method, also known as the Gruber-Widal test, became the forerunner of specific serum diagnosis. The appreciation of the fact that in a disease like typhoid fever, in which often the most experienced clinicians had failed in differential diagnosis, a positive diagnosis could be made with a drop of blood and a culture of typhoid bacilli, served to make the method extremely popular and the fact that agglutination is not of the diagnostic value in all infectious diseases, cannot detract from its historical and actual importance.

Soon after Widal's application of the agglutination test to [illegible] was studied for diagnostic purposes

[illegible] Therapy. Philadelphia [illegible]

[illegible] Wash.: [illegible]

[illegible]

[illegible] & Wilkins Co.

in tuberculosis, especially by Arloing & Courmont,[6] and then by many others, amongst them by ourselves. The outcome of these studies of the reaction in the diagnosis of tuberculosis was not as satisfactory as it had been found in enteric fever, because of the positive results observed with sera from persons who presented no clinical evidence of tuberculous disease, a fact which was made use of by Arloing to emphasize the frequency of latent tuberculosis or of tuberculous infection; and also because of negative results especially in far-advanced cases of manifest tuberculosis. These irregular findings caused numerous observers to attach but a limited or no value to the reaction for diagnostic purposes; others, among them ourselves, found it of corroborative significance to other methods, especially when the reaction was found positive in higher serum-dilutions. Arloing & Courmont accepted a positive reaction with serum dilution of 1:5 as an indication of the presence of tuberculosis. Later studies showed, however, that a positive agglutination in itself, even in higher dilutions, could not be utilized in any case for the DIAGNOSIS OF TUBERCULOUS DISEASE, and we ourselves found that the sera of clinically healed cases were often positive in dilutions of 1:20 and even 1:40, and that in cases of tuberculosis, under specific treatment with the Watery Extract of Tubercle Bacilli, the titer could be raised to ten or more times that which had been present before treatment. Nevertheless we still believe that a positive reaction in serum dilutions of 1:10, and over, can aid in the diagnosis of certain obscure cases.

Robert Koch[7] published his observations of the increase of the agglutination-titer in sera from patients treated with Tuberculin [BE] (Bacillus Emulsion), and his observations caused him and others to look upon such an increase as an evidence of immunity against the tubercle bacillus, although he expressed himself adversely in regard to the diagnostic value of the reaction. Koch's view seemed to us justifiable on finding that our clinical results were the better, the higher the agglutinating titer was on the final discharge of the patients, and, in the

[6] ARLOING & COURMONT; 4e Congrès de la Tuberculose; Paris, 1898, 583 and 586.—Zeitschr. f. Tuberkulose, 1900, I, 11.—Deut. med. Woch., 1900, XXVI, 766.

[7] ROBERT KOCH; Deut. med. Woch. 1901, XXVII, 829.

Winyah Report, published in 1907, we tabulated a series of 200 cases studied in this light. Since that time other reactions have become available as indices of specific immunity, and the studies of sera in the new light indicate that we were only partly correct in our view, and that the agglutinating titer is not a quantitative index for immunity, because this can be complete with a low agglutinating titer; also that its increase, as we observed it, was the result of the continued administration of the Watery Extract in favorably selected cases which had acquired a maximal degree of antibacterial immunity in the earlier period of their treatment.

While thus the agglutination-reaction cannot be accepted as a quantitative test for immunity, it serves as a diagnostic test in so far as it indicates that a contact with the related antigen has occurred at some prior time. Agglutinins are, however, believed to be a provision of defense against infection in so far as they probably may act as an aid to phagocytosis and as they may, in the case of certain bacteria, tend to inhibit their motility and possibly their multiplication.

Like the precipitin-reaction to be considered immediately, the agglutination-reaction is characterized by the fact that it occurs in the presence of the specific antibody alone, without the assistance of complement.

PRECIPITATION

The reaction of precipitation occurs when an immune serum is added to a filtrate of its related bacterial culture or to a bacterial extract; or in other words, when an immune serum is added to its specific antigen in solution. The reaction is specific and is characteristic in that it often occurs in very high dilutions of immune serum, while it may be negative with low dilutions of the same specimen of serum. The substance which causes the precipitation is called the *precipitinogen* and is contained in the antigen; the precipitable substance, the *precipitin*, is derived from the immune serum.

Precipitating substances can be produced with vegetable as well as with animal proteins, and the reaction permits the distinction of different kinds of proteins in a far-reaching degree,

making possible, for instance, the differentiation of horse serum from rabbit serum. Its specificity is limited to its occurrence in high dilutions and, in this event, it is of diagnostic importance. Its greatest practical value lies in its application for forensic purposes in which it serves for the differentiation of blood-stains, for the discovery of sophistication in food-stuffs (horse-meat, etc.), and for many other purposes.

Like agglutinins, precipitins are a provision for defense against infection, and are possibly analogous reaction-products, formed in response to the parenteral digestion or cleavage of foreign proteins. According to Kolmer, they may be concerned in preparing their antigens for destruction and solution, that is, for the bactericidal and bacteriolytic action of the immune serum.

The precipitins were discovered by Kraus[8] in 1897, and are closely related to the agglutinins.

THE OPSONINS

It has been mentioned that Metchnikoff was obliged to recede from his first position which attributed to the phagocytes a practically exclusive rôle in the organic resistance to bacterial infection. This necessity became particularly urgent, when it was shown that leucocytes which had been washed completely free from all traces of serum, retained at best a very slight phagocytic power and that, therefore, phagocytosis depended upon the cooperation of antibacterial serum-substances. Metchnikoff accordingly acknowledged (1893) that the body-fluids, especially of immune animals, contained certain substances which facilitate phagocytosis by stimulating the leucocytes to ingest the bacteria. These substances he called *stimulins*. Later researches, first by Denys & Leclef (1895), then by Leishman, and especially by Wright & Douglas,[9] showed, however, that these serum-substances do not act in the manner of "stimulins" of leucocytes, but rather by weakening or otherwise influencing the bacteria in some way, in consequence of which they become

[8] R. KRAUS; Wien. klin. Woch. 1897, X, 736.—1901, XIV, 1016.

[9] A. E. WRIGHT & S. R. DOUGLAS, in WRIGHT; Studies in Immunization. London, 1909, 75.

more readily subject to ingestion by leucocytes. These serum-substances were therefore called *opsonins* by Wright (1902) (from *opsono*, to prepare for a meal, to render palatable).[10]

Following Denys & Leclef, Neufeld & Rimpau also studied the phagocytic power of the blood, independently of Wright, and found similar substances which they called *bacteriotropins*. Neufeld[11] claims that bacteriotropins are different from opsonins, although most authors believe them to be identical with what is now known as thermostable opsonins, or immune opsonins.

If leucocytes are washed free from serum and are added to a thin emulsion of bacteria, for instance, tubercle bacilli, only a few of the latter are taken up by the leucocytes. If, however, serum is added to the mixture of leucocytes and bacteria, or if the bacteria are first acted upon by the serum and then exposed to the action of the leucocytes, it will be found that the latter take them up readily, showing that ingestion takes place under the influence of certain serum-substances upon the bacteria.

The opsonins of the normal serum are thermolabile; they are destroyed by heating to 56° C. for one-half hour and by standing. The serum of persons, who have acquired an immunity against a certain virus, contains opsonins which are specific for this virus and which have a more powerful action than the normal opsonins, from which they also differ in being heat-resistant or thermostable.

The opsonic power of a serum, to a certain bacterium, is ascertained by determining the average of bacteria which are ingested by a certain number of leucocytes; the result was designated, by Wright, as the *phagocytic index*. On comparing the phagocytic index of a person infected with a certain bacterium, with that of a healthy person, the *opsonic index* of the patient

[10] Much, who believes that the opsonic action is a phenomenon identical with the bactericidal action and that opsonins are identical with bactericidins, assumes that the action of the antibody may stop short of actual lysis, only a portion of the bacterial protoplasm being made to exude ("Ausschwitzung" of Baumgarten), and that such partially exuded bacterial substances exert a particular attraction or positive chemotaxis upon phagocytes.

[11] F. NEUFELD; in KOLLE & WASSERMANN, Handbuch, II. Ergänzungsband; Jena, 1909, S. 303.

is obtained. Thus, the phagocytic index of the patient (for instance 4) divided by the phagocytic index of the healthy person (for instance 5) gives an opsonic index of 4:5 or 0.8.

An opsonic index which is stationary between 0.8 and 1.1 is considered as an evidence of health; a lastingly high or low index as diagnostic of disease, while a variable index is believed to indicate a deficient immunity. The opsonic index can be raised by specific treatment with a vaccine of the related bacterium, and, in tuberculosis, Wright employs by preference Koch's Tubercle Bacillus Emulsion which he administers in very small and slowly increasing doses. The injection of a dose is followed within a few hours by a sudden fall in the opsonic index, which is called the *negative phase* and may persist for several days or longer, after which the index again rises, reaching a point above the previous figure in favorable cases. A dose of vaccine, given on the downward grade of the opsonic index, is apt to increase and prolong the negative phase, the reverse being observed if the vaccine is administered on a rising index.

In explanation of the many unfortunate results observed during the first tuberculin-era, Wright suggested that some of the very large doses of tuberculin, which were then given, were probably administered during the downward grade of the negative phase.

The observation of the negative phase was believed to be of importance, because, according to Wright, the patient is in a susceptible condition for the time being, and because therefore a dose of vaccine during the negative phase might be productive of harm. It was then claimed that the daily determination of the opsonic index would furnish a useful guide for the administration of the specific remedy, but the routine determination of the opsonic index for the purpose of regulating the doses and the intervals between them, has not been found practicable, for the reason that the technic of the test is too complicated and difficult and that it requires too much time. The fear that specific treatment in any infectious disease, but especially in pulmonary tuberculosis, might be dangerous at the hands of the general practitioner or wherever the control of the opsonic index was not feasible, does not appear justified, and

Emery [12] states that patients may improve remarkably even during a prolonged negative phase, so that a low index does not necessarily prove a lack of immunity. One of the most striking cases of amelioration of a severe case of tuberculosis, which he has ever seen, occurred during a negative phase lasting over three weeks.

Observations of the opsonic index have shown that cycles of negative and positive phases follow upon one another even without specific treatment, especially under the influence of exercise. It was suggested in explanation that exercise or exertion stimulates the tuberculous focus and that then an increased absorption of specific toxins takes place, which causes an increased formation of specific antibodies, the whole representing a process of *auto-immunization*. This phenomenon has been studied particularly by English physicians, and is claimed to be a necessary condition for healing in all cases of infection with the tubercle bacillus and of tubercle formation, in which progressive disease does not develop or is arrested spontaneously. The method has been employed largely by Dr. Paterson of the sanatorium at Frimley,[13] who observed improvement following far more rapidly in patients who are prescribed work which is graded according to the degree of "auto-inoculation" induced thereby. He noted a more rapid decrease in sputum, gain in weight and general improvement, in patients who had been set to work under careful supervision, than in those who followed the rest-cure. As a means of preventing the development of phthisis, the stimulation of this auto-immunization is not without merit, and its importance is insisted upon, for instance, by Hort [14] who declares that no amount of general treatment is of the slightest avail in the treatment of a chronic disease like tuberculosis, in the absence of specific treatment, whether this be autogenous or artificial. In persons who recover without artificial immunization, the "specific treatment" can, in Hort's opinion, only have been supplied by unrecognized auto-immunization. Aside from Paterson, this form of immunization has

[12] W. D'Este Emery; Immunity and Specific Therapy. New York (Hoeber), 1911, 280.

[13] Marcus Paterson; Brit. Med. Journ. 1909, II, 1055.

[14] E. C. Hort; Rational Immunization in the Treatment of Tuberculosis. New York (Wood), 1909, 19 ff.

been studied and applied in practise especially by Freeman, Meakin, Wheeler and Inman.

In studying the nature and origin of the opsonins, the question arose whether the opsonins, which form and increase during specific treatment, are identical with the normal opsonins. We have already called attention to some essential differences between the two, and will add that Wright himself and most other authors deny their identity. It being the thermostable kind of opsonin which develops and increases after the administration of a vaccine, many authors have expressed the opinion that the "immune opsonins" are identical with amboceptors. In support of this view it has been pointed out that the two substances, opsonin and amboceptor, arise under the same conditions and are identical in their power of resisting heat, in their facility of combining with bacteria, and in their specificity. An equally close resemblance appears to exist between thermolabile opsonins and complement; both occur in normal serum and are destroyed by heating to 56° C.; both are nonspecific.[15]

In our examinations[16] of sera of tuberculous persons under specific treatment, we found the curves for opsonins and those for amboceptor to show a marked parallelism, in that a high titer of amboceptor and a high opsonic index usually coincided, whereas when the complement-fixation was negative, or positive only in traces, the opsonic index was low. These curves differed materially from that of the agglutinins in non-treated patients, which appears to show only slight variations, but increases steadily under the influence of specific treatment; they differ also from the curve of the precipitins, which rises and falls inversely to those of opsonins and amboceptors.

[15] The question has been raised whether there are two kinds of thermostable opsonins, one acting with, and the other without complement, the former being identical with the humoral bactericidins, the latter with the leucocytary bactericidins. In respect to this, Much has found that, in some cases, in the same patient the opsonic power of the plasma is greater than that of the serum, but he believes that the question cannot be settled at the present time.

[16] K. v. Ruck & S. v. Ruck; Journ. Amer. Med. Assoc. 1910, LIV, 954.

CHAPTER IV

BACTERIOLYSINS AND BACTERICIDINS. THE COMPLEMENT-FIXATION REACTION

We have heretofore referred to the fact that certain bacteria cause their injurious effects upon the animal organism chiefly through a ferment-like substance produced by active secretion, which is called a true bacterial toxin, whereas others manifest their pathogenic action mainly through products derived from their body-substances, which are called endotoxins; and that both, true toxins and endotoxins, can be employed for artificial immunization against their related antigens. The secretion within the tissues, or the parenteral introduction of a true toxin causes the formation of an antitoxin, and a similar production or introduction of an endotoxin stimulates the formation of an antibacterial antibody, a substance which is antagonistic to the endotoxin and to the living bacterium from which this has its origin. This antibody has been called *bacteriolysin* or *bactericidin,* because of its power to bring about the disintegration, i. e., the parenteral digestion of the related endotoxin or of the living bacterial cell, and to destroy its virulence. The complement-fixation test has for its purpose the macroscopic demonstration of the presence, in a certain serum, of the specific substance which gives rise to this bacteriolytic or bactericidal action and which was first demonstrated *in vivo,* in 1894, in the study of the peritoneal exudate of an animal previously immunized against cholera and then infected, by intraperitoneal injection, with cholera vibrios.

Pfeiffer [1] showed that, if a suspension of a culture of cholera vibrios is injected into the peritoneal cavity of a guinea-pig which has been actively immunized against cholera, it is possible to study the fate of the vibrios, by examining at intervals a drop of exudate removed from the peritoneal cavity by means of a glass capillary. In his experiments he found that

[1] R. PFEIFFER; Zeitschr. f. Hygiene. 1894, XVIII, S. 1.

the vibrios first lost their motility and that their outlines became less sharp; then they broke up into granules, and eventually they disappeared altogether; all this WITHOUT the intermediation of phagocytic cells. Pfeiffer's explanation of this phenomenon [2] thereafter was extended by Bordet [3] who found in his investigation of the mechanism of the reaction, that two substances are concerned in the action of the immune serum upon the bacterial cell; one, a thermolabile component which is present not only in fresh immune serum, but also in fresh normal serum; this he identified with Buchner's *alexin*, retaining the same name for it; and another, a thermostable component which is specific and appears in response to treatment with a related antigen. Bordet concluded from the results of his experiments that the thermostable, specific substance prepares or "sensitizes" the bacterial cell for the lytic ferment-action of the alexin; he therefore called it *substance sensibilatrice* or *sensitizer*.

Bordet arrived at his conclusions through the study of *hemolysis*. It had been known for some time that the normal serum of some animals has the power of dissolving the red blood-cells of another species, hen-serum, for instance, being hemolytic for rabbit-cells, guinea-pig serum sometimes for calf-cells, and human serum for sheep-cells. Observations of this kind had, among others, been made by Belfanti and Carbone,[4] Ehrlich,[5] Landsteiner,[6] v. Dungern,[7] and analogies had been drawn between this hemolytic action and the bactericidal power of normal serum. It was Bordet [8] who discovered that, by treating an animal with parenteral injections of red blood-cells or with tissue-cells of another species, substances are formed in the blood-serum of the treated animal, which act specifically against the foreign cells, in a similar manner as the bacteriolytic antibodies of immune serum act against their related bacteria, and which can be demonstrated *in vitro;* these substances were

[2] R. PFEIFFER; Deut. med. Woch. 1896, XXII, 97.

[3] J. BORDET; cf. Studies in Immunity, translated by F. GAY. New York (Wiley), 1909, 56.

[4] BELFANTI & CARBONE; Giorn. d. R. Acad. di Torino; 1898, cf. M. HAHN, in KOLLE & WASSERMANN, Handbuch, IV. 1. 1904. S. 284.

[5] P. EHRLICH; Immunitätsforschung. Berlin, 1904.

[6] LANDSTEINER; Centrlbl. f. Bakt. 1899, XXV, 546.

[7] v. DUNGERN; Münch. med. Woch. 1899, XLVI, 405.

[8] J. BORDET; Ann. Institut Pasteur. 1898, XII, 688.

called *hemolysins.* Their discovery gave rise to many investigations of the more complicated processes and problems of antibacterial immunity. They resulted in the further development of the side-chain theory by Ehrlich, in its application to the phenomena of antibacterial immunity. One of the earliest results of the discovery of specific hemolysins was the *complement-fixation reaction.*

Bordet recognized the parallelism between the phenomena, which he had observed in these experiments, and those in bacteriolysis of the cholera vibrios, and showed that both are due to the cooperation of two substances, sensitizer and alexin; he applied his results to the elucidation of the process of bacteriolysis, as it was observed in Pfeiffer's experiment, claiming that the alexin which exerts its ferment-action upon the foreign cells, whether erythrocytes or bacterial cells, was the same substance in either case.

A similar explanation was proposed by Metchnikoff who called the specific thermostable substance *fixateur,* and the non-specific thermolabile component *cytase.* In support of his theory of cellular immunity, he held, however, that the disintegration of the bacteria, although shown by Pfeiffer to occur without phagocytosis, was nevertheless of leucocytary origin, the substances concerned in the process acting only after they had been liberated through the breaking up of the phagocytes, that is to say, after *phagolysis.* Metchnikoff was able to show that, when phagolysis was prevented, the phenomenon did not occur as described by Pfeiffer, but that, instead, the bacteria were taken up and were digested by the phagocytes.

In numerous experiments, some of which we have described heretofore, we found that the active blood-serum of immunized persons or animals caused marked morphological changes upon tubercle bacilli *in vitro,* the bacilli being reduced to a fine granular detritus. A like reduction appeared to occur *in vivo,* which was exclusively intracellular, after injecting the tubercle bacilli into the peritoneal cavity of a fully immunized guinea-pig.

In studying the phenomenon first described by Pfeiffer, and the experiments and conclusions of Bordet tending to its explanation, Ehrlich [9] applied the principles of his side-chain theory

[9] P. Ehrlich; Immunitätsforschung, *loc. cit.*

of immunity, which he had first laid down in 1897.[10] He arrived at different conclusions from those of Bordet, in regard to the mode of action of the two substances responsible for the process of bacteriolysis or of hemolysis. As we shall refer to the side-chain theory again, we will mention here only that, in Ehrlich's opinion, the thermostable component of immune serum, or the sensitizer of Bordet, is attracted to the bacterial or the blood-cell, and that then it attaches to itself the thermolabile substance or alexin. When this union is completed or, in other words, when the alexin has been bound or fixed to the bacterial or to the blood-cell, through the intermediation of the sensitizer, it, that is the alexin, exerts its ferment-function upon the cell and causes its disintegration. In accordance with this view, Ehrlich [11] calls the sensitizer *amboceptor* because it unites with two substances, and he designates the alexin as *complement* [12] because it complements or completes the reaction. Since the side-chain theory impresses us as affording the best and most probable explanation of the processes of immunity, we shall adhere to its nomenclature and shall therefore employ Ehrlich's terms of amboceptor and complement exclusively, in our subsequent discussions.

It is generally assumed that the antibacterial action in the immune organism, like the hemolytic action, constitutes a process of digestion, in the course of which the foreign bacterial or other cells, or their substances, are made to undergo cleavage on entering the interior of their host; their protoplasm-protein is dissociated into simpler elements which are ultimately assimilated or excreted.

Just as in hemolysis no actual and complete solution of the cell occurs, but the stroma of the red blood-cell simply gives up its hemoglobin, the frame substance remaining behind as a "shadow," so the bacterial cell likewise does not dissolve completely, but loses its protoplasm. Sometimes "shadows" of tu-

[10] P. Ehrlich; Wertbestimmung des Diphtherieheilserums, 1897; cf. Immunitätsforschung, S. 73, *loc. cit.*

[11] P. Ehrlich & J. Morgenroth; Berl. klin. Woch. 1901, XXXVIII, 251.

[12] Various other terms have been proposed for these two substances, but in order to avoid confusion, we prefer not to mention any of them, the more so as those of Ehrlich are in most common use and are universally understood.

bercle bacilli may be seen under the microscope on examination of "lytic" slides, when decided morphological changes have occurred in the tubercle bacilli under the influence of the fresh immune serum.

In the case of many bacteria, the bacteriolysis *in vitro* takes place quite readily under the influence of active immune serum, but others, for instance, the bacilli of anthrax and of pest, and especially the acid-fast bacilli oppose considerable resistance. In the case of the tubercle bacillus, the occurrence of bacteriolysis *in vitro* has been denied, although the experiments of some authors, and also our own, appear to show that, with the fresh serum of a highly immunized person or animal, marked morphological changes can be produced upon tubercle bacilli, and that the latter lose their virulence during their incubation with the serum in connection with the experiment. Citron [13] asserts that the amboceptor-action of fixing or binding complement in the presence of the specific antigen must not be identified with the bacteriolytic action, and considers it necessary to differentiate between cytolytic (bacteriolytic) amboceptors and those which bind complement.

A similar difference suggested itself to us in 1912, in certain experiments with immune sera, which had been inactivated by heat, before the addition of virulent tubercle bacilli for the bacteriolytic experiment *in vitro*. The amboceptor-content of the sera had been high, as was shown by the complement-fixation test, but no lytic action could be observed on the addition of fresh serum from a normal animal or of fresh serum from a presumably normal person. The animals, which were infected with the contents of these tubes, acquired tuberculosis, the same as did the controls. We concluded that this suggested the presence, in the active immune serum, of a specific component which is thermolabile and is destroyed by inactivation.

When these experiments were repeated the following year, the animals, contrary to our expectation, did not acquire tuberculosis whereas the controls died of generalized tuberculous disease. These last experiments being more numerous and being to all appearance perfectly conducted and well observed, we concluded that the results of our first experiments must be

[13] J. CITRON; Die Methoden der Immunodiagnostik und Immunotherapie. Leipzig, 1912, S. 166.

set aside for the time being, and that the bacteriolytic and bactericidal action of inactive serum was completed by phagocytosis, or by the action of the complement of the normal animal, after it had been infected with the content of the lytic tube.

Before we could spare the time to repeat the experiments again, an explanation of these unexpected results appeared to be given when it was found that the thermometer, used in the water-bath in which the immune sera were inactivated, gave excessive readings, registering four degrees higher than was actually the temperature of the water-bath. The sera in the last experiment had therefore not been heated to 56° C. and had not been inactivated completely, or possibly not at all.

A repetition of these experiments was now urgently required, and it was made in April, 1914, with 14 specimens of inactivated immune serum and 6 specimens of active immune serum. This time our first observation was confirmed, in that all animals infected with 0.01 mgr. of virulent tubercle bacilli after incubation with inactivated immune serum, and all controls infected with the same dose of the same tubercle bacillus emulsion after incubation with normal serum or with normal salt solution, presented typical generalized tuberculosis; whereas the six animals for the infection of which the tubercle bacilli, from the same emulsion and in the same dose, were incubated with the immune sera in their fresh, active state, were found entirely free from tuberculosis, when all animals were killed and autopsied, fifty-one days after the infections had been made.

Under the circumstances the conclusion was unavoidable that the heating of these sera to 56°–57° C. for thirty minutes destroyed some specific substance, probably of the nature of a specific ferment, which the organism elaborates in response to the infection.

In view of the impossibility of reactivating these sera for the bacteriolytic and bactericidal action, by the addition of complement, it is of interest to mention that such a reactivation was possible for the action of the opsonins.

The following table gives our observations upon six immune sera, of the complement-fixation test, and of the differences in the opsonic index and the bacteriolytic action noted between fresh immune serum and inactivated immune serum, also the result of attempted reactivation, upon the opsonic index and

the bacteriolysis, by the addition of normal complement from various sources:

Immune Serum	Complement-fixation Complete With				Opsonic Index			Bacteriolysis				
									Inactive Serum, Reactivation Attempted With			
	T. B. Emulsion	Neutral Fats	Fatty Acids	Protein	Active Serum	Inactivated Serum	Inactive Serum +G.P. Compl.	Fresh Active Serum	Human Serum	Calf Serum	Rabbit Serum	G.-Pig Serum	Normal Salt Sol.
								Complement Control					
Human Serum #1.....	1:8	1:4	1:4	1:4	1.3	0.96	1.3	2+	0	0	0	0	0
" " #2.....	1:4	++	++	++	1.2	0.32	1.2	1–2	0	0	0	0	0
" " #3.....	1:4	1:4	1:4	1:4	1.2	0.6	1.2	2	0	0	0	0	0
" " #4.....	1:8	1:4	1:4	1:8	1.4	0.55	1.35	2	0	0	0	0	0
Calf " #1.....	1:4	1:4	1:4	1:4	1.	0.4	0.95	1–2	0	0	0	0	0
" " #2.....	1:4	1:4	1:4	1:4	0.98	0.3	0.86	1+	0	0	0	0	0

The complement-fixation test became available through the studies of Bordet & Gengou, who applied the results of their studies in hemolysis, and the observed analogy with the process of bacteriolysis. These authors[14] subjected the red blood-cells of a rabbit to the action of inactivated serum of an animal of another species, which had been immunized with washed red cells of a rabbit, and thereafter added fresh guinea-pig serum as complement. The red cells were dissolved as had been expected. To this mixture they then added cholera vibrios which had been subjected to the action of inactivated cholera immune serum, that is to say, which had been "sensitized." If any complement was available in the solution, they expected that the sensitized cholera vibrios would take up or fix this, and would then undergo bacteriolysis. No change was observed, however, in the vibrios because all the complement had disappeared from the fluid, having been used up or "fixed" by the red cells in the earlier reaction. When, on the other hand, the order of the experiments was reversed, and sensitized cholera vibrios were mixed with complement, permitting this to be fixed, and when the sensitized red blood-cells were then added to the mixture, no hemolysis took place, because the complement had disappeared, having been used up and bound by the bacteria.

It is evident that the reaction of hemolysis was peculiarly adapted as an aid in the ocular demonstration, in a given serum, of specific amboceptor for its related bacterial antigen, inasmuch as, when a properly adjusted amount of complement was present, it became used up and disappeared. If no amboceptor was present for the antigen, or if the antigen was left out, or in case it had no relation to the specific amboceptor, the complement remained free and unimpaired in the test tube and was available for the second experiment in which hemolysis then took place.

Having assembled in previously determined proper amounts the heated or inactivated immune serum, and the bacterial antigen against which it is to be tested, and the normal serum which supplies the necessary complement, the tube containing the mixture is kept, for one to two hours, at the proper temperature which favors their union, that is, 37° Centigrade

[14] Bordet & Gengou; Ann. Institut Pasteur, 1901; cf. Bordet-Gay, *loc. cit.*

(98.6° Fahrenheit). If then the previously heated or inactivated serum of an animal, which has been immunized against certain red blood-cells, is added and likewise a suitable quantity of the same kind of red cells, but no complement, the solution of the red cells occurs only if complement is still available by being left over from the first test. In this case, the blood-cells dissolve and give up their hemoglobin to the otherwise colorless content of the tube. The conclusion is that no specific amboceptor for the particular bacterial antigen was contained in the tested serum, because the complement had not disappeared but could be used for the hemolysis. If, on the other hand, the red cells remain intact and settle to the bottom of the tube, the content of which then remains colorless, this shows that no complement was available for their disintegration, and we assume that the serum which we tested did contain a specific amboceptor which, together with the complement, united with the bacterial antigen or, in technical language, that the complement was fixed to the antigen by the specific bacterial amboceptor.

By means of this reaction it was shown that the sera of animals that had been immunized against the typhoid bacillus, the plague bacillus and the like, as well as sera from persons who had recently recovered from typhoid fever, contain specific amboceptor for these bacilli, whereas none could be shown to be present in normal sera.

At first the reaction aroused only an academic interest, as being of assistance in scientific research. In 1903, Bordet & Gengou [15] employed the method for the purpose of showing that guinea-pigs, treated with subcutaneous injections of avian tubercle bacilli, soon form an amboceptor in their blood, which is capable of fixing complement in the presence of, not only avian, but also human tubercle bacilli as antigens.

Gengou [16] made use of the method to demonstrate specific amboceptors in the sera of animals which had received injections of albuminous substances in solution, such as milk, albumin, serum; he found that complement is fixed on the addition of

[15] Bordet & Gengou; cf. Bordet-Gay, *loc. cit.*, p. 462 (p. 89).

[16] Gengou; C. R. Soc. d. Biol., 1906, LVIII, 218, and Bordet-Gay; *loc. cit.*, 464. Berl. klin. Woch., 1906, XLIII, 1531, and Bordet-Gay; *loc. cit.*, 467.

the specific antiserum to the antigen. The discovery that a protein in solution could serve as an antigen, was developed further by Neisser & Sachs, and especially by Wassermann & Bruck [17] who studied the reaction with sera from tuberculous subjects, and showed that tuberculin and bacterial extracts, or extracts from diseased tissues could be employed with equally satisfactory results as those obtained with bacterial emulsions. In their further studies, the authors reversed the reaction, as had been done by Neisser & Sachs, by testing extracts of tuberculous tissue against sera known to contain specific amboceptors, having been tested with positive results against tuberculin as the antigen. The positive results obtained in the complement-fixation test with sera of persons treated with tuberculin, caused them to designate the specific amboceptor, which they demonstrated with tuberculin for the antigen, as *antituberculin*.[18] Gengou [19] published further observations in which he showed that treatment of guinea-pigs with human and avian

[17] WASSERMANN & BRUCK; Med. Klinik, 1905, I, 1409. Deut. med. Woch., 1906, XXXII, 449.

[18] It will be well to point out that old tuberculin is a mixture of the proteid substances contained in the culture medium and of specific substances produced by tubercle bacilli, during their growth upon it, and that it (tuberculin) immunizes the organism against a variety of proteid substances. The antibodies formed in response to the injection of old tuberculin are, therefore, not all specific for tubercle bacilli or for their several toxins.

For this reason, old tuberculin is not an acceptable antigen in the technic of the complement-fixation test, as an index of specific immunity to the tubercle bacillus, since it contains substances which are not derived from the bacillus and which give rise to reaction-products of their own. It is further to be noted that the chemistry of the tubercle bacillus, although studied very imperfectly as yet, indicates that its composition is a very complicated one, and that therefore the amboceptors, which are capable of binding complement in the presence of tubercle bacillus antigens, must possess correspondingly manifold affinities. While we are not assured that the fractional or partial antigens of the tubercle bacillus, as far as they have been isolated by ourselves and by others, are chemically pure, and therefore are actually the true partial antigens, we are convinced that the principle involved in these researches is correct and that an immunity, as indicated by a positive complement-fixation, is complete only in so far as the amboceptors for the individual constituents of the tubercle bacillus can be shown to be present, in optimal amounts, in the immune serum. This subject will receive further consideration in Chapter X.

[19] GENGOU; cf. BORDET-GAY, *loc. cit.*, p. 467 (p. 89).

tubercle bacilli, and with acid-fast bacilli in general, may give rise to the formation of amboceptors which are active for the various types of mammalian tubercle bacilli, and that in general the injection of acid-fast bacilli, in guinea-pigs, causes the formation of amboceptors which are active against other acid-fast, and particularly against human, bovine and avian tubercle bacilli.

A practical application of the Bordet-Gengou reaction was undertaken by Widal & Lesourd who employed it for the diagnosis of typhoid fever. Using an emulsion of typhoid bacilli and the serum of typhoid fever patients, they found that a positive reaction occurred more frequently and earlier than in the agglutination test. These observations represented probably the first direct use of the test for diagnosis, but it was not until several years later that the diagnostic possibilities of the method were inquired into further.

It was known that the complement-fixation test may serve to demonstrate either the presence of an antigen, as was done by Neisser & Sachs and by Wassermann & Bruck, if a known antibody is present in the serum, or it may show the antibody, as was done in the original reaction, if a known antigen is present. Both applications of the method depend upon the fixation of the complement, as it is indicated by the inhibition of hemolysis, when red blood-cells and hemolysin (hemolytic amboceptor) are added. In either mode of application, the test was found useful for the diagnosis of a variety of infectious diseases, both human and animal. We believe, however, that it is an error to assume that a positive result in the complement-fixation test for the demonstration of specific amboceptors, necessarily indicates the presence of active disease. Occurring in response to the absorption of bacteria or of their toxins, the presence of specific amboceptor indicates a defensive reaction on the part of the infected organism, tending to destroy them. This defense can manifest itself in various degrees of efficiency. As it remains active after the infection is overcome, for shorter or longer periods of time, during which the reaction continues to be positive, the demonstration of the specific amboceptors is diagnostic only in the sense that an infection has occurred at a previous period. In case specific amboceptors are found in the course of an infectious disease, they are diagnostic

of the nature of the infection, and indicate at the same time that the defensive apparatus of the organism has become mobilized and is in action.

In the case of a bacterial invasion which results in local tissue-degeneration and necrosis, as it occurs in tuberculosis, the spontaneous development of specific bacteriolytic amboceptors against the bacteria themselves takes place too late, as a rule, to protect the organism against serious damage, while the presence of such amboceptors against toxic products which the bacteria secrete in their living state, affords the organism no other protection than that of preventing symptoms caused by them.

It may likewise happen, in the course of an infectious disease, that no specific amboceptors are demonstrable in the serum of the patient. This may be because the function of their production has not yet been stimulated sufficiently, or because the bacterial invasion or the toxemia are in excess of that for which the defense is adequate. In either case the absence of amboceptors is not diagnostic, but rather prognostic and therapeutic in its import; in the former case it is an indication for specific stimulation, and in the latter case for measures which prevent the excessive absorption of the bacteria and of their toxins, or in case of an infection with the so-called true toxin-bacteria, it is an urgent indication for the administration of the corresponding antitoxin.

We shall have occasion to consider this subject further in the chapter on the practical application of specific remedies in the prevention and treatment of tuberculosis; our reference to it here has the object of showing that a positive or negative reaction in complement-fixation, of sera when tested for specific amboceptors against the related *bacterial* antigens, need have no diagnostic import whatever. Much greater importance attaches, in our opinion, to a positive result of the test, when it is reversed for the purpose of demonstrating the specific antigen, which would then indicate the presence, in the tested serum, of the related bacteria or of their toxins.

The most important outcome of these diagnostic studies, was the application of the complement-fixation test for the diagnosis of syphilis, by Wassermann, Neisser & Bruck,[20] in the reaction

[20] WASSERMANN, NEISSER & BRUCK; Deut. med. Woch. 1906, XXXII, 745.

commonly known as the *Wassermann Reaction* which, as we shall see, is not specific in the sense that it demonstrates the presence of related amboceptors to the virus which causes syphilis.

THE WASSERMANN REACTION

In the adaptation of the complement-fixation test for the diagnosis of syphilis, by Wassermann, Neisser & Bruck, no specific antigen was available, because the Treponema pallidum had not been cultured successfully; but in accordance with their results with extracts from tuberculous organs, the authors first employed extracts of syphilitic organs for the purpose. In the course of the study of the reaction, a great variety of organic extracts and of other substances was investigated for their antigenic properties, and it was found that extracts from certain normal tissues served equally well as antigens for syphilitic reaction-products for the binding of complement, and these non-specific extracts have since replaced, more or less, those made from the livers of infants who had died of congenital syphilis. The significance of a positive result of the complement-fixation test in syphilis, when made with a non-specific antigen, can therefore under no circumstances indicate the presence, in the serum, of a specific amboceptor against the microörganism which stands in relation to the disease. It is not our purpose, nor relevant to our subject, to discuss the Wassermann reaction at length; this has been done in many excellent textbooks on the subject and concerns us only incidentally. It may, however, be mentioned in passing, that more recently Noguchi [21] has used a specific complement-fixation test in syphilis in which he employs a pure pallidum-extract as an antigen, having succeeded in culturing the virus artificially. On examining the sera of animals immunized with the pure pallidum-extract, using for antigens both specific pallidum-extract and non-specific lipoids, he found that these immune sera bound complement with the pallidum-extract but not with the lipoids. On the other hand, sera from rabbits with active experimental syphilitic orchitis bound complement with the lipoids but not with the pallidum-extract

[21] H. Noguchi; Serum Diagnosis of Syphilis. Philadelphia, 1912, 3rd edition, p. 159.

These findings show that the immune sera contain the specific antibodies for the pallidum but not for the lipoids, and that, in the sera of syphilitic rabbits in the active stage of the disease, there is an abundance of the lipotropic substance capable of rendering complement inactive, by fixation, in the presence of certain non-specific lipoids, but not enough of true specific amboceptors to bind complement with the specific pallidum-antigen. This experience completely demonstrates the difference between the "Wassermann" and the true antibody-antigen reaction in syphilis.

THE COMPLEMENT-FIXATION TEST IN TUBERCULOSIS

It was quite natural to apply the complement-fixation test to the study of tuberculous disease, and we employed the method soon after Bordet & Gengou's publication in 1903. Our results were, however, so irregular that we did not feel justified in continuing the work; but we resumed our studies after the experiences of Wassermann & Bruck had been published in 1906. These authors were soon shown to have been in error in supposing that complement-binding substances appeared only in the blood-serum of persons who have been treated with tuberculin, as was shown first, among others, by Lüdke,[22] S. Cohn,[23] Citron,[24] Wolff-Eisner,[25] and by our own comparatively large experience in over ten thousand individual examinations, made in our laboratory since 1906, which gave ample evidence that complement-fixation may be positive with sera from tuberculous persons who have not been treated with tuberculin or any other specific product of the bacillus of tuberculosis.

In the further investigation of the reaction, by many authors, it was found that it was often negative in the early cases of tuberculous disease. Wolff-Eisner [26] obtained a greater proportion of positive results in early cases, when he used several

[22] H. LÜDKE; Münch. med. Woch. 1908, LV, 783. Beitr. z. Klin. d. Tub., 1907, VII, 47.

[23] S. COHN; Beitr. z. Klin. d. Tuberkulose. 1908, XI, 143.

[24] J. CITRON; *loc. cit.*, S. 173 (p. 92).

[25] WOLFF-EISNER; Frühdiagnose und Tuberkulose-Immunität. Würzburg (Kabitzsch), 1909, p. 249.

[26] A. WOLFF-EISNER; *ibid.*, S. 65.

different antigens (old tuberculin; new tuberculin; emulsion of tuberculous tissue). Further, the reaction was found to be positive in the sera of persons suffering with other diseases, and in cases of healed tuberculous disease, so that, in course of time, the value of the complement-fixation test for the diagnosis of clinical tuberculosis very naturally became doubtful.

The fact that the complement-fixation reaction can be positive with the serum of persons who are not clinically tuberculous, was interpreted, wrongly we believe, as arguing against its specific character. We believe with Fritzsche [27] that a positive qualitative test indicates, in the same manner as does a positive cutaneous tuberculin test, that the reacting person has experienced an infection with tubercle bacilli at some time in the past;[28] but in addition it also indicates that his organism has responded with the development of a certain degree of immunity. The observation of the reaction in clinically non-tuberculous persons, who are affected with other diseases, does not controvert this view, since, with the great frequency of tuberculosis-infection, it is a matter of course that persons giving positive reactions to tubercle bacillus-antigens should occasionally be affected with other diseases; and this fact does not in the least disprove the specific character of the reaction; on the contrary, it confirms it.

The fact that the test may be positive in human subjects who have not been treated specifically, regardless of the presence of clinical tuberculous disease, is what should be expected especially with low serum-dilutions or with the undiluted serum. We have frequently observed this result in our studies of supposedly normal sera or of sera taken from tuberculous individuals.

When the reaction is positive qualitatively, the test may be interpreted in the same manner as is the cutaneous tuberculin

[27] Ernst Fritzsche; Inaug. Diss. Zürich, 1908.

[28] This does not, of course, apply in the case of normal individuals, who neither react to tuberculin nor show complement-binding substances in their sera, but who develop the latter after immunization with a suitable vaccine. The appearance of complement-binding substances in response to vaccination of non-tuberculous persons supports the view that complement-fixation with the specific antigen is an immunity-reaction (see also Much, in Brauer, Schröder & Blumenfeld; Handbuch der Tuberkulose; Leipzig, I, 1914, S. 300, ff.).

test. It is different, however, with the quantitative test, as it was practised, among others, by Christian & Rosenblat,[29] Engel & Bauer,[30] Jochmann & Möllers[31] and, from the beginning, by ourselves. The higher the dilutions of the immune serum are, in which complete fixation of the complement can be obtained, the more of the specific antibody, which causes the union of complement and antigen, must necessarily be present in the serum of the individual or animal which is tested. The quantitative method can therefore be used as an index for the amount of specific amboceptor contained in the serum. In applying this measure as an index of immunity, we found that animals, previously treated with vaccine, resisted a subsequent infection only in those cases in which the titer of the complement-fixation test was high, and also, that this titer could be raised to an effective degree by continued treatment, in the human subject and in the experiment animal. Although positive QUALITATIVE reactions were observed frequently in guinea-pigs and rabbits after one or two small doses of our vaccine, these animals failed to resist infection; sera from human subjects also failed, as a rule, in manifesting a complete germicidal action unless the titer in complement-fixation corresponded with that found in the sera of the resisting animals. Under these circumstances we have found the test useful clinically as well as in our experimental studies.

Analogous observations have been made in the case of diphtheria-antitoxin in the sera of persons who have never had clinical diphtheria. Wassermann[32] and Abel[33] showed years ago the presence of specific antibodies to the diphtheria bacillus in the blood of healthy persons, and Otto,[34] as well as other authors, declares that the serum of human newborn infants almost constantly contains diphtheria antitoxin. It disappears during the first year of life, and then reappears and gradually increases from decade to decade, being present in 63 per cent of persons

[29] CHRISTIAN & ROSENBLAT; Münch. med. Woch. 1908, LV, 2032.

[30] ENGEL & BAUER; Münch. med. Woch. 1908, LV, 2273.

[31] JOCHMANN & MÖLLERS; Veröffentl. d. Robert Koch-Stiftung. Berlin, 1912, H. 3.

[32] A. WASSERMANN; Deut. med. Woch. 1894, XX, 120, V.

[33] RUD. ABEL; Deut. med. Woch. 1894, XX, 899.

[34] R. OTTO; Deut. med. Woch. 1914, XL, 542.

forty years of age. In persons who never had diphtheria, this specific immunity, which is often sufficient for protection, may result from repeated infections with small quantities of diphtheria bacilli or with bacilli that are not, or only slightly virulent, as was suggested many years ago by Bretonneau.[35] An effective, spontaneously acquired immunity, i. e., one without preceding diphtheritic disease, is found particularly in persons who have been in contact with diphtheria for some time, as in nurses and other attendants in diphtheria-wards.

That a very decided immunity can develop without specific treatment, is demonstrated by a case reported by Kleinschmidt,[36] of a male infant, ten months old, with diphtheria of the oral mucous membrane and, more particularly, of the anal region. The virulence of the infection was shown by the fact that diphtheria bacilli isolated from the anal lesion killed a guinea-pig in two days. This affection healed spontaneously, the patient receiving no treatment whatever, beyond the customary hygienic measures. On discharge, seventeen days after admission, his blood was found to contain ten antitoxin-units per cubic centimeter of blood, a quantity which is unusually large even after active immunization.

These experiences are in accord with and support our contention, and also verify our observations that a complete and effective active immunity to the tubercle bacillus can develop, not only in response to artificial immunization, but also spontaneously, by way of response to repeated infections which are not severe enough to produce disease; or, if the disease has developed, that the resulting immunity may become sufficient to overcome it.

Quantitative determinations of the amboceptor-content are made by successive reductions of the amount of serum which is to be tested. Thus, if 0.05 cc. of the tested serum has caused the disappearance of all the complement, we can try half that amount, and we may reduce it still more, until we reach a minimal amount of serum with which a disappearance of the complement no longer occurs. If therefore all other conditions are the same, and we still find the reaction positive, in that all the

[35] BRETONNEAU; cited by E. v. BEHRING: Diphtherie. Berlin (Hirschwald), 1901, S. 184.

[36] H. KLEINSCHMIDT; Münch. med. Woch. 1913, LX, 1477.

complement has disappeared from the tube which contained only one-fourth or one-eighth of a drop of the immune serum, this shows that there was a larger amount of specific amboceptor in it than was necessary to fix all the complement when a whole drop or one-half drop was used, and we can thus express the amboceptor-content of the immune serum by the fractions of one drop of the undiluted, or of a diluted serum, which still cause the complete disappearance of the complement; or, unless we choose to state directly the amount of immune serum, we can simply give the denominator of the fraction, that is, 4-8-16, etc., instead of 1:4; -1:8; -1:16.

In our own work, we begin as a rule with 0.05 cc. of immune serum, and express incomplete results by one or more checks, accordingly, as more or less complement appears to have disappeared; if all had disappeared with this or with a higher fraction and an incomplete disappearance is shown with the next higher fraction, we add the check to the fraction with which the disappearance was complete.

It may be mentioned with respect to the technic of the complement-fixation test, that not all tubercle bacilli show the same avidity to combine with the specific amboceptor or to form as firm a union with it as do others, and that this may be influenced by the age of the same culture or by the state of the emulsion; probably other conditions, which are more or less obscure and which alter the results, are also of importance. The adjustment of the hemolytic system likewise requires great care in the preliminary tests, one fruitful source of trouble being the observation that serum which is used for complement is occasionally found to possess a spontaneous hemolytic power for the blood-cells used in the test. In our experience, a hemolytic amboceptor of very high titer may manifest a stronger attraction for complement than does the combination antigen plus bacterial amboceptor plus complement, and it may break up the union of the complement in this combination, thus "deviating" it. It has also come to our observation that a specific immune amboceptor appears to unite with complement without any antigen being present and that in this manner the reaction may become obscured. Finally the serum to be tested for specific amboceptor may of itself be capable of absorbing complement, and unless this has been ascertained on a preliminary examination

and is compensated by the addition of more complement, the test will of course be unreliable.

It will be seen therefore that the technic of the complement-fixation test is quite complicated, consisting as it does of two distinct reactions into which five separate substances enter normally. Although the several components are always standardized or titrated separately, although control tubes are put up, and all reasonable care and precautions are observed, yet anomalous and contradictory results may make their appearance, the cause or causes of which in a given instance may remain obscure, and it may be very difficult indeed to discover them. It would serve no good purpose to discuss, in this connection, all possible causes of irregular reactions, which may contribute in obscuring the results; in fact, such a discussion would only be confusing. Those who are conversant with the laboratory technic know and appreciate these possible sources of error; for the general practitioner it suffices to obtain an understanding of the principles of the reaction as described above, because it is not serviceable in his routine work as a practical test, but belongs to the laboratory and should be done by specially trained workers.

While the principles of the test remain the same, different laboratories vary more or less in technical details, on account of personal experience or preference, or as may appear desirable in the studies of immunity in individual infectious diseases.

The theory of the complement-fixation reaction will become elucidated further by considering it in the light of the side-chain theory of Ehrlich.

THE SIDE-CHAIN THEORY OF EHRLICH

It has already been said that, in his investigation and study of the reactions which we have described, in their relation to immunity, and in an attempt to find an explanation for the *modus operandi* of immunizing processes, Ehrlich applied the principles of his famous side-chain theory which had originally been advanced in 1885 for the purpose of explaining the processes of nutrition, and had been elaborated and extended, in 1897, so as to account for the phenomena of antitoxic immunity which, like those of antibacterial immunity, in the last instance

represent digestive processes. In contrast to Metchnikoff's physical conception of resistance, Ehrlich's explanation is based on chemical phenomena. It is important not to lose sight of the fact that Ehrlich's theory, as well as all other attempts to explain the related observations are theories, that is to say, hypotheses by which the mechanism of certain observed phenomena may be explained, and that they have been advanced with no other purpose; least of all have they been offered as actual facts. It is not without justice that Bordet asserts that all these hypotheses have been taken too much for granted and that their hypothetical nature has been lost sight of. Whatever may be the actual truth, however, in the present state of our knowledge the side-chain theory serves excellently as a guide for the manner in which we may assume the processes of immunity to unfold themselves.

The side-chain theory assumes, in analogy to the side-chains of the benzol-ring, that the protoplasmic cell (red blood-corpuscle, bacterial cell, etc.) has certain "side-chains" or receptors, that is to say, affinities which attract molecules that may serve for its nutrition. Substances which are introduced parenterally and enter the circulation, as for instance toxins, are attracted to cells which have suitable receptors for them. The molecule of a true toxin is believed to have a double function, or two functionating "groups," one being that by which it can be attached to the cell and which "fits" to a particular receptor of the cell, the *haptophore group* (*hapto*, to touch), and the other, the *toxophore group* which is the bearer of the specific toxic property. If now the toxin-molecule is attracted to, and unites with its particular cell-receptor, this receptor, being occupied, can no longer functionate and the cell may perish either through the toxic action intermediated through the toxophore group, or for want of further sustenance. In accordance with a natural law, however, which is known as Weigert's Law, the cell develops new receptors and discharges the one which is occupied by the toxin-molecule. The new receptors are produced in excess and cannot all remain attached to the cell; they are therefore discharged into the surrounding fluid (the circulation) and are ready to unite, by means of their haptophore groups, with toxin-molecules which have entered the circulation, and to neutralize them, thereby preventing them from be-

ing attached to, and injuring the body-cells. When free in the circulating tissue fluids, these receptors are called *antibodies*, the toxin is neutralized by its union with the antibody, and the antibody for a true toxin is therefore an *antitoxin*. This represents the simplest aspect of the process. Ehrlich designated the receptors which, on being discharged into the circulation, become antitoxins, as receptors of the first order.

By a somewhat more complicated, but still relatively simple process, the receptors of the second order are produced, which are concerned in agglutination and precipitation. These receptors of the second order are cast off from the cells in like manner as the antitoxins, and are considered as having two "groups" or affinities, viz., a haptophore group by which they attach themselves to the cells against which their action is specific, and a *zymophore group*, which is the bearer of their specific function and is, in the case of agglutinins, responsible for what Ehrlich calls the process of coagulation (*Gerinnungsvorgang*).

It is to be noted that the action of the receptors of the second order, as also that of the third order, is not directed solely against soluble substances like the toxins, but also against foreign cells (bacteria).

The process becomes still more involved when larger molecules of foreign proteins, lipoids, alien tissue-cells, or bacteria, enter the tissues parenterally. If the tissue-cells have fitting receptors, these are occupied and cast off similarly as in the case of true toxins: but for the disposal of atomically more complex and more highly organized substances, it is necessary that they be prepared for assimilation and, instead of a simple neutralization, as it takes place in the case of true toxins, their actual breaking up or cleavage is prerequisite in order to reduce them to that simple and elementary state in which the organism can make use of them. In order to accomplish this, the specific amboceptor, as represented by the cast-off receptor, requires the cooperation of ferment-like substances, the complements, which have a proteolytic or a lipolytic action.

The amboceptor must unite not only with the foreign protein- or cell-substance, but also with the complement, and it therefore requires two haptophore groups, one being the specific *cytophile group* by which the amboceptor is attached to the

cell, and the other the non-specific *complementophile group* by which it anchors or binds the complement. It is therefore through the intervention of the specific amboceptor that the complement can attack and reduce the foreign protein or cell-molecule into a simpler molecular state and thus fit it for assimilation. Ehrlich designates *amboceptors* as receptors of the third order.

The antibodies of the third order are discharged by the tissue-cells as receptors formed in excess in response to cell-stimulation which may be purely physiological, but is in greater part specific, viz.: stimulated through the parenteral introduction of certain alien substances. These antibodies anchor or unite with the foreign substance by means of one of their haptophore groups; with the other group they anchor or bind the ferment-bearing complement. The disintegration of the foreign substance becomes possible through the union of the three.

This brief description attempts to give the teachings of the side-chain theory in so far as they are required for an elementary understanding of antitoxins, hemolysins and bacteriolysins.

In order to make the side-chain theory applicable to all phenomena which presented themselves in the course of these studies, it was found necessary to account for apparently anomalous observations which required additional investigation and specially planned experiments. The outcome of these further researches led to and supported the theory of the multiplicity of complement, of anti-complement, of the deviation of complement, also of the multiplicity of amboceptors and anti-amboceptors. While these and other supplementary demonstrations in support of the side-chain theory, by Ehrlich and his coworkers, are of great interest and importance in serological studies, we omit their consideration as apt to prove confusing to those who are not special students in immunology. For the analysis and explanation of these and many other details, a study of the publications in support of, or in opposition to Ehrlich's theory is necessary.

For the reader who desires to extend his studies, translations of the principal publications of Ehrlich,[37] of Bordet,[38] and of

[37] P. EHRLICH; Collected Studies on Immunity. Translated by Bolduan. New York (Wiley), 1910.

[38] J. BORDET; *loc. cit.* (p. 89).

their coworkers are available, and they will also find them considered in the textbooks on the subject of immunity by Emery,[39] Zinsser,[40] Kolmer [41] and others. The large handbooks by Kolle & Wassermann [42] and by Kraus & Levaditi [43] have not been translated and are needed only by students who are engaged in practical and experimental investigation.

In spite of their different view-points, or possibly because of them, Ehrlich and Bordet have done more to further and to develop the study of immunity, in recent years, than any other scientists. It may be said that they never differ in principle, the same facts and phenomena being admitted by both observers. Their differences lie only in the interpretation of these facts, and even here they are not as far apart as may appear to the casual student.

As one of the results of all these studies, we find that while, after ordinary ingestion, foreign protein- or bacterial substances, which the organism can utilize in nutrition and building-up of tissue, are prepared and converted by the digestive organs to that simple elementary form in which they are suitable for assimilation, the organism possesses a similar function for their preparation and conversion in its physiological interior, in the event that such foreign substances accidentally find admission through other than the ordinary channels. This additional function becomes operative through stimulation, by the foreign substances, of certain cells, and the latter must, in Ehrlich's view, possess receptor-groups which can unite with corresponding groups of the former. In the case of such a union, the foreign substance becomes subject to the action of the ferments of the blood and is then reduced in a similar manner as in the ordinary course of gastro-intestinal digestion. The stimulation of the cells having taken place, their receptors are produced more plentifully and, becoming detached from the

[39] EMERY; *loc. cit.* (p. 86).

[40] HANS ZINSSER; Infection and Resistance. New York (Macmillan), 1914.

[41] JOHN A. KOLMER; *loc. cit.* (p. 80).

[42] KOLLE & WASSERMANN; Handbuch der pathogenen Mikroorganismen. Jena, 1903-1909.

[43] KRAUS & LEVADITI; Handbuch der Technik und Methodik der Immunitätsforschung. Jena, 1908-1909.

cells, they appear free in the circulating blood and are ready to unite with like foreign protein- or bacterial substances which, by accident or design, may find repeated access to the tissues or directly to the blood.

On its first occurrence, the reduction of foreign substances, in the tissues or blood, appears to be slow and gradual, taking place step by step, and the resulting intermediary products may cause no symptoms, especially when the amount of the foreign substance introduced is small. When the cleavage occurs rapidly, because the organism is sensitized on account of like reactions having occurred previously, and especially when in such a case the amount of the foreign substance is large or is introduced directly into the blood, or if it is absorbed rapidly from serous membranes like the peritoneum or pleura, then these intermediary cleavage-products are likely to be present in sufficiently large amounts to cause toxic phenomena to which small animals are apt to succumb.

On prolonged disuse of the function, because the access of the particular foreign substance to the blood is not repeated, the free specific antibodies in the blood may gradually disappear; but they reappear much more promptly than before when they are again needed because of a later repetition of the cause that gave rise to their formation in the first place.

If, after its introduction, a foreign substance finds no fitting receptors in the cells with which it comes in contact, or if it is not of such a nature that it can serve the organism as food, no digestive or reducing reaction can occur; its influence upon the organism, and its destiny then depend upon its particular nature. Inert substances may be eliminated if they are in solution; if they are present in solid form, the tendency is to encapsulate, or to remove and deposit them elsewhere by phagocytes. Against poisons like morphine, strychnine, arsenic, the organism is defenseless, regardless of the mode of introduction.

The disposal of non-pathogenic bacteria is the same as would follow the parenteral introduction of other foreign formed elements.

Proteid substances in solution elicit a more prompt reactive response to their parenteral introduction than do suspensions in a corpuscular or finely divided solid state. This is true particu-

larly when the administration is subcutaneous, and it becomes important in attempts at immunization with bacterial emulsions or with bacterial products which are already in solution.

It is evident from the foregoing considerations that the immunization of the organism, or the establishment of specific immunity to a pathogenic bacterial invasion is a process in which the organism is "trained" to develop those protective substances which render the action of the bacteria innocuous, whether this finds its expression in the secretion of toxins, or through the effect of the endotoxins, or of other injurious bacterial substances.

Under favorable conditions the process of immunization takes place spontaneously in response to the bacterial invasion and it may become effective in causing healing, or in preventing the bacteria from exerting their pathogenic action in a maximal degree. In cases in which the spontaneous response of the organism is quantitatively insufficient, its defense can be supplemented by supplying it with the related antitoxic or antibacterial substances from another immunized organism, or the ability of the organism to defend itself may be increased by direct stimulation with the related bacteria or with their respective toxins. In this case successful artificial immunization against pathogenic bacteria is practicable even in an organism that is not yet infected by the bacteria against which an immunity is to be produced, for the purpose of protecting it against a subsequent infection. This is done by means of the related virus in a form in which it is not injurious, of itself, to the organism and in which it is readily broken down; or even better, in a form in which it is broken down already into its stimulating or antigenic constituents, in solution. Active prophylactic immunization has been practised for more than a century against smallpox, and for a decade against typhoid fever, the virus of both diseases apparently being easily disintegrated in the vaccinated organism.

It is our purpose to show in the practical and experimental parts of this volume, that active immunization can be applied successfully also against tuberculosis, and that the prophylactic immunization or vaccination against the tubercle bacillus is simple and practical, as well as without danger, if certain conditions are complied with.

PART II

PRACTICAL IMMUNIZATION AGAINST TUBERCULOSIS

CHAPTER V

SPECIFIC PROPHYLACTIC IMMUNIZATION AGAINST HUMAN TUBERCULOSIS

HISTORICAL

The attempts at specific prophylactic immunization against tuberculosis date back to the discovery of the tubercle bacillus in 1882. Soon after Koch's first publication, Falk [1] wished to determine whether tuberculosis belonged to those infectious diseases in which one attack protects against the further acquirement of the disease. In his endeavor to secure an attenuated virus, in analogy to the attenuated smallpox virus employed by Jenner for his classical example of specific prophylactic immunization, Falk infected guinea-pigs with tuberculous tissues which he had attenuated by putrefaction. Later virulent infection did not meet with an increased resistance in the experiment animals, and Falk came to the conclusion that successful prophylactic vaccination against tuberculosis, corresponding with Jennerization against smallpox, was *a priori* improbable.

Like investigations received a strong impetus when Koch announced his tuberculin in 1890, in which the tubercle bacillus-toxin was believed to have been discovered and by means of which it was hoped that protective immunization would become possible. Even before the announcement of tuberculin by Koch, an American physician, Dr. Samuel G. Dixon, the present Commissioner of Health of Pennsylvania [2] had succeeded in protecting guinea-pigs against virulent infection, by treatment with avirulent and atypical cultures of tubercle bacilli, in which club-shaped, branched and other forms predominated.

Although a specific immunization against the tubercle bacillus cannot be produced by means of old tuberculin, as has been shown amply by van Calcar, Hans Aronsohn, Sahli and others,

[1] F. Falk; Berl. klin. Woch. 1883, XX, 772.

[2] S. G. Dixon; Med. News; 1889, Oct. 19; cf. Reprint, Med. and Surg. Reporter, 1890, Sept. 6.

its therapeutic employment made possible undoubtedly better clinical results than had been secured without its aid, and, in the cases in which it was used persistently for prophylactic purposes upon infected children, especially by Petruschky, certain benefits were apparent, even though these could not be attributed to a direct specific immunization against the tubercle bacillus. The reason for this can now be understood and lies in the ability of tuberculin to stimulate the tuberculous focus and thereby to arouse the self-protective mechanism of the body. Since tuberculin can exert such an action only upon the infected, or upon the actually tuberculous organism, any therapeutic effect, to which it may give rise, is directed against the progression of the disease; it cannot be active in the direction of preventing the development of a tuberculous focus from an infection.

The result of Koch's own subsequent attempts, induced by his modified views, to obtain more satisfactory therapeutic results from Tuberculin TR and his Bacillus Emulsion or Tuberculin BE, finally led him to express the opinion that an effective immunity against the tubercle bacillus could be produced only by means of a living virus, suitably attenuated, and this opinion guided the originators of various methods of prophylactic immunization of cattle, the history of which can be found in most textbooks on immunity and on the specific treatment of tuberculosis. An excellent résumé of these attempts was given quite recently by Gilliland.[3] The principal methods are those of Koch & Schütz's Tauruman; of v. Behring's Bovovaccin; of Klimmer's Anti-Phymatol, and the collodium-sac method of Heymans.

In the prophylaxis of human tuberculosis, Koch's opinion formed the basis of Friedmann's investigations with human tubercle bacilli attenuated spontaneously by passage through the turtle organism, and of those of Webb and Williams who claim to have secured a satisfactory immunity in monkeys and in two children, by their method of injecting definite numbers of counted virulent tubercle bacilli, commencing with the injection of a single microörganism. Both these methods are too

[3] S. H. Gilliland; The Production of Artificial Immunity against Tuberculosis in Cattle. State Livestock Sanitary Board, Harrisburg, Pa. Circular No. 32, 1915.

greatly fraught with danger ever to become practical, nor could they be adopted by the general practitioner. In Friedmann's method the infiltration and frequent abscess formation at the site of injection are also serious disadvantages.

In his first publication on tuberculin [4] Koch touched upon one phase of specific prophylaxis when he said: "The new method can only then become a blessing to suffering mankind, when as many incipient cases of tuberculosis as possible are treated, and when therefore severe advanced cases no longer occur, such as now form an inexhaustible source for new infections."

As early as 1891, Heubner [5] suggested that in cases of hereditary predisposition, tuberculin might be useful prophylactically. In 1903, v. Behring [6] proposed a passive immunization of nurslings by means of the milk of immunized cows; but aside from the fact that this method is not quite without danger, since Weber & Titze [7] have demonstrated virulent tubercle bacilli in such "immune" milk, Hamburger [8] showed that it was not practicable because it would not serve to transmit unchanged antibodies except during the very first few weeks of life. In his report at the Cassel meeting of German Naturalists and Physicians, v. Behring (*loc. cit.*) expressed the hope that his researches would finally make the sanatoria and other institutions superfluous, by means of the same method by which Jenner had made the old pest-houses unnecessary.

In the same year (1903), Maragliano [9] announced, at the XIV International Medical Congress at Madrid, a vaccine, the nature of which he did not describe, with which he could actively immunize animals against virulent infection with tubercle bacilli, and produce antitoxic, antibacterial and agglutinating substances in the human organism. He declared it a mistake to assume that an active immunization was possible only through the injection of living cultures of tubercle bacilli, and claimed

[4] Robert Koch; Deut. med. Woch. 1890, XVI, 1029.

[5] Heubner; Berl. klin. Woch. 1891, XXVIII, 452.

[6] E. v. Behring; Deut. med. Woch. 1903, XXIX, 689.

[7] Weber & Titze; Tuberkulose-Arbeiten a. d. kais. Gesundheitsamte. Berlin, 1907, Heft 7, S. 9.

Titze; *ibidem.* 1908, Heft 9, S. 50.

[8] F. Hamburger; Beitr. z. Klin. d. Tuberkulose. 1905, IV, 23.

[9] E. Maragliano; Berl. klin. Woch. 1903, XL, 563.

that v. Behring's recent assertion to this effect would, if it were true, preclude *a priori* the possibility of immunization against tuberculosis in man. At the International Tuberculosis Conference in Berlin, in October, 1913, Maragliano again referred to his method which he had extended for prophylactic purposes and Dr. Enrico Castelli [10] presented a paper at the recent meeting of the NATIONAL ASSOCIATION, at Seattle, Washington, June, 1915, in which he described the continued success of Maragliano's method.

Of other recent attempts in the direction of a practical immunization, those of Möller and of E. Klebs may be mentioned, who made use of the principle of attenuation by passage through a non-susceptible organism, viz.: that of the blind-adder; also those of Calmette who claims that a sufficient degree of immunity can be produced by the oral administration of tubercle bacilli attenuated by culturing on oxgall. J. Bartel claimed, in a communication to the Washington Tuberculosis Congress [11] to have given proof that it was possible to initiate a successful method of specific immunization against tuberculosis by subjecting tubercle bacilli to the action of certain tissues, especially of lymphoid tissues.

In the meanwhile the researches of Deycke in lepra, and his preparation of Nastin had stimulated work in the direction of employing the body substances of the tubercle bacillus, including its fats and waxes for the purpose of securing an active immunization. These attempts were the more rational since a corpuscular vaccine, consisting of living or killed tubercle bacilli, cannot be expected to stimulate an immunizing response on the part of the normal organism, until the bacterial protoplasm is brought in solution and probably also broken down.[12] Accordingly, Deycke,[13] Much and their coworkers [14] investigated

[10] ENRICO CASTELLI; Trans. 11th Annual Meeting, National Association, Seattle, Washington, 1915, p. 211.

[11] J. BARTEL; Trans. Washington Tuberculosis Congress; 1908, I, 1, p. 188.—Wien. klin. Woch.; 1905, XVIII, 1144, and elsewhere.

[12] cf. WRIGHT, MORGAN, COLEBROOK & DODGSON, cited by A. FLEMING; The Practitioner; 1914, XCII, 420.

[13] DEYCKE PASHA & RESCHAD BEY; Deut. med. Woch. 1907, XXXIII, 89.

[14] G. DEYCKE & H. MUCH; Münch. med. Woch., 1909, LVI, 1985; 1913, LX, 119, and elsewhere.

the immunizing effect to be secured by solutions of the tubercle bacillus in various organic solvents, and these studies culminated in the separation of Much's *Partial Antigens,* that is, preparations of the tubercle bacillus dissolved in weak organic acids. Töniessen [15] attempted to produce immunity by means of autolyzed cultures and succeeded, in guinea-pigs and mice, with those which contained all partial antigens. Borissjak, Sieber & Metalnikow [16] found that after immunization with tubercle bacillus-wax, and with defatted tubercle bacilli, antibodies were formed not only against the tubercle bacillus-waxes, but also against living and dead tubercle bacilli. These investigations emphasize the importance of employing a complete antigen in solution for the purpose of securing a complete immunity against the tubercle bacillus in the human subject.

Mention must also be made of Weleminsky's *Tuberculo-mucin,* by means of which he and others secured excellent therapeutic results, and with which he has recently undertaken the prophylactic immunization of children.

Weleminsky started with the intention of isolating, from the culture-fluid used for the preparation of old tuberculin, that substance which would prove to exert a healing effect in guinea-pig tuberculosis, however slight this effect might be. Instead of working up the culture-fluid of six-weeks' old cultures, as is customary in the manufacture of old tuberculin, he permitted the cultures to grow much longer, preventing the evaporation of the glycerin-bouillon by employing culture-flasks which permitted only a slight access of air. In this manner many successive generations of tubercle bacilli were obtained on the same medium and, after about one year's growth, certain cultures showed a slight tendency to induce healing in tuberculous guinea-pigs. By selective culturing, Weleminsky perpetuated the particular cultures which showed this tendency and a substance was developed in the medium, in the course of several years, which gave the reactions characteristic of a mucin, and which Weleminsky calls *Tuberculo-mucin,* attributing to it the healing properties of tuberculin.

In selecting those cultures which were to be perpetuated,

[15] E. Töniessen; Berl. klin. Woch. 1914, LI, 93.

[16] Borissjak, Sieber & Metalnikow; Zeitschr. f. Immunitätsforsch. Orig. 1912, XII, 65.

Weleminsky paid no attention to their effect in causing the formation of antibodies, but solely to their influence upon the tuberculous process in guinea-pigs, believing that the formation of immune substances is not identical with the healing effect.

The mucin to which Weleminsky attributes the healing action was developed in increasing quantities in the culture-medium by his selective culturing, and is still increasing constantly in these cultures.

Tuberculo-mucin is not toxic and does not injure tuberculous guinea-pigs even in considerable doses. The culture-medium, however, and also the residual culture-bouillon, after the mucin has been extracted from it, both kill tuberculous guinea-pigs, showing that they contain tuberculin, the toxic principle of the culture-medium upon which tubercle bacilli have been grown. Weleminsky employs for his therapeutic uses both the "original culture-fluid" and the pure tuberculo-mucin.

Animal experiments in guinea-pigs with experimental tuberculosis and in cattle with spontaneous tuberculosis were successful in so far as, in the former, the development of tuberculosis could be arrested, or that it could be prevented if the tuberculo-mucin was administered at the time of the infection. The healing effect was marked in tuberculous cattle, even in moderately advanced disease. Weleminsky states that his attempts to produce a protective immunization against later experimental infection have, so far, not been successful, although they promise results against the acquirement of spontaneous stable-infection in cows.

Several clinical reports have been published on the results of the use of tuberculo-mucin in human tuberculosis, first by Pachner [17] and then by several other clinicians, which are reviewed by Weleminsky in his latest collective report.[18] In pulmonary, as well as in other forms of tuberculosis, very encouraging therapeutic results were obtained, in all of which the essential rôle of the focal reaction upon the healing processes is manifested.

Tuberculo-mucin is diagnostic in the production of the characteristic local and focal reactions; it is also claimed to be

[17] E. PACHNER; Beitr. z. Klin. d. Tuberkulose; 1912, XXV, 137.—Zeitschr. f. Tuberkulose; 1914, XXI, 529.

[18] F. WELEMINSKY; Tuberculosis; 1914, XIII, 456.

prognostic in so far as cases which are not capable of responding to the stimulation, and therefore show no focal reactions to the injection of the remedy, have been found to terminate fatally, while in all those cases in which focal reactions were observed a favorable course of the disease ensued.

Weleminsky's tuberculo-mucin possesses a great interest in many respects; first, in the increase, by selective culturing, of a particular substance which appears to exert a decided healing action upon the tuberculous guinea-pig organism; then in the fact that this substance is not toxic and is, in fact, entirely distinct from the toxic principle of tuberculin; further in the fact that, while it promotes healing, it does not appear to manifest an immunizing action in the sense that a protection against subsequent experimental infection is produced.

The specific nature of tuberculo-mucin is evidenced in its healing action upon the tuberculous organism, and in the fact that it produces focal reactions; it is further shown in the influence which it exerts upon obscure conditions which must be attributed to a tuberculous intoxication, such as is observed in various manifestations: for instance, in "tuberculous rheumatism," in some skin affections, and in other ways.

The results of our chemical and experimental studies of the constituents of the tubercle bacillus (Chapter X, p. 285) make it appear probable that the tuberculo-mucin of Weleminsky is identical with a heat-coagulable protein which we have isolated and which is necessarily lost in the manufacture of old tuberculin when it is concentrated by heat.

The favorable results of Petruschky and of Crofton with the use of preparations which they believe to contain the complete antigen, especially the fatty constituents of the tubercle bacillus, have been considered in another connection. While these authors have accomplished their successes only after prolonged treatment, their results, as well as those in the general application of specific treatment of tuberculosis, show that the human organism responds to the parenteral introduction of specific products of the tubercle bacillus in a favorable manner, which must be attributed to the development or increase of an active immunity against the specific virus. (See p. 133 and p. 135.)

Much additional evidence could be collected from literature to show the successful protection of lower animals, even of

small laboratory animals like guinea-pigs; in young cattle the value of prophylactic vaccination is no longer denied or even questioned seriously. We have never doubted that the human subject would respond to vaccination in like manner as has been observed in cattle, providing a vaccine were employed which contained all body-substances of the bacillus in an uninjured form, and providing that a sufficiently large dose could be administered with safety. This desideratum, we believe, we have complied with by the methods in which we prepare our vaccine, and we have overcome the necessity of prolonged treatment or of intravenous administration by removing the excess of lipoids which cause local necrosis.

As far as we have been able to determine, our method, elaborated through many years of study and research, is the first in which a complete and effective immunity of the normal human organism against the tubercle bacillus and against tuberculosis has been produced by means of the administration of a single dose, in the manner of Jennerization, and by which infected and also early, that is to say, closed, uncomplicated and non-febrile cases of tuberculous disease have been apparently cured or arrested by means of one, two or three doses. After a period of nearly five years since we began the practical application of our vaccine for prophylactic purposes, we and others have observed nothing that requires a modification of our earlier opinion, namely, that the results are uniform and are obtainable with absolute safety and with sufficient simplicity of application that the method may be employed by the general practitioner.

STUDIES LEADING TO OUR PRESENT METHOD

Our studies with old and with purified tuberculin, in their therapeutic and prophylactic effects, date back many years during which the value of these remedies was investigated also experimentally upon laboratory animals. In these experimental studies, however, the desired uniformity of success, which we deemed necessary in order to justify positive claims, was not possible of attainment at that time, although clinically the aid derived from the remedies was sufficiently manifest to cause

us to persist in their employment, after the medical profession had practically abandoned them as useless and even dangerous.

Greater improvement in our clinical results than had been witnessed before with old tuberculin, was observed in 1896 after the administration of a purified product made by extracting the bacilli in their culture-medium, and still better results were secured with the adoption, in 1897, of a watery extract of tubercle bacilli prepared from bacilli that had previously been freed of their fats.[19] Animal experiments with this latter preparation also proved to be more encouraging; but it still appeared to us that the results were not sufficiently uniform and, on that account, the experiments were never published. While we succeeded more frequently than formerly in demonstrating an apparent protective or therapeutic effect, the failures in parallel and simultaneous experiments were still too numerous; further animal experimentation with the Watery Extract was then abandoned, while our studies were continued in the search for a more effective preparation.

Several series of bactericidal experiments with sera of apparently recovered patients, made during the years from 1899 to 1906, failed completely. The causes for these failures can now easily be explained; they were evidently due to the fact that we sterilized the immune sera on three successive days at 56° C., and that we incubated excessive amounts of tubercle bacilli with the immune sera which had been selected with reference to their agglutinating titer but without regard to their content of other specific antibodies.

In the light of our subsequent experiences, and especially of those accumulated during the several years past, the older experiments with the Watery Extract of Tubercle Bacilli have acquired a new interest for us and possibly also for others, and the relation of a few of them, in this place, may be justified as showing what encouragement they gave for continuing the work. Studied in the light of later experiences, they also suggest to us the probability that, apart from insufficient treatment to account for our failures, the reading and interpretation of autopsy results may have been more or less obscured, even at that time,

[19] KARL v. RUCK; Therapeut. Gazette. 1897, XXI, 338. Trans. Amer. Climatol. Assoc., 1897, XIII, 74.

by certain pseudo lesions which we had not learned to differentiate.

Experimental Treatment with Watery Extract of Tubercle Bacilli Before and After Infection

GUINEA-PIG No. 247. August 21, 1896. Weight 605 Grams.

Treated before infection with Watery Extract, until October 12, 1896. Received 40 doses, from 0.2 cc. to 0.5 cc. each, of the 0.01 per cent solution of the Watery Extract; a total of 18.3 cc., representing 1.83 mgr. of the dried extract.

October 13, 1896; weight 600 gms. Infected subcutaneously with 0.1 mgr. of virulent tubercle bacilli.

Treated after infection, from October 14, 1896, to April 26, 1897, with 95 doses, 0.5 cc. each, of the 0.01 per cent solution of Watery Extract; a total of 42.5 cc. representing 4.25 mgr. of the dried extract.

Died May 3, 1897. Weight 560 gms., having lost 40 gms.; lived 201 days after infection.

Autopsy: Infection-point, healed scar. Regional lymph-glands fibrous. Liver greatly enlarged; tubercle bacilli in smears from crushed tissue. Spleen enlarged; macroscopic tubercle, but no tubercle bacilli in smears from crushed tissue. Lungs contain one fibrocaseous tubercle, only one single tubercle bacillus being found in smear. Other organs normal.

GUINEA-PIG No. 248. August 21, 1896. Weight 530 Grams.

Treated before infection, and infected the same as No. 247. Weight 600 grams.

Treated after infection, October 13, 1896, to January 10, 1897, with 45 doses of 0.5 cc. each, of the 0.01 per cent solution of Watery Extract, a total of 22.5 cc., representing 2.25 mgr. of dried extract.

Died January 12, 1897. Weight 490 gms., a loss of 110 gms., 89 days after infection.

Autopsy: Infection-site caseous; regional and other lymph-nodes caseous; very few tubercle bacilli in smears. Liver and spleen enlarged, fatty degeneration and doubtful tubercle; very few tubercle bacilli in smears. Lungs, isolated fibrous nodules; only two tubercle bacilli found in smears from crushed tissue.

GUINEA-PIG No. 249. August 21, 1896. Weight 345 Grams.

Treated before infection, and infected the same as guinea-pig No. 247. Weight 415 grams.

No treatment after infection.

Died April 17, 1897. Weight 430 gms., a gain of 15 gms., 185 days after infection.

Autopsy: Infection-site, minute caseous spot; inguinal lymph-glands enlarged, not caseous; no tubercle bacilli found in smears from crushed tissue. Liver very much enlarged; fatty degeneration and tubercle; few

acid-fast fragments in smears from crushed tissue. Spleen, few large caseous foci; no tubercle bacilli found in smears. Lungs, small, in some places coalescent nodules; no tubercle bacilli found in smears.

GUINEA-PIG No. 250. August 21, 1896. Weight 860 Grams.

Treated before infection with 34 doses, from 0.2 to 0.6 cc. each, of the 0.01 per cent solution of Watery Extract, a total of 16.1 cc., representing 1.61 mgr. of dried extract.

Infected the same as guinea-pig No. 247. Weight 745 gms., a loss of 115 gms. during treatment.

No treatment after infection.

Died December 25, 1897. Weight 550 gms., a loss of 185 gms. in 74 days after infection.

Autopsy: Infection-site caseous; few tubercle bacilli in smears. Regional and other lymph-glands caseous; few tubercle bacilli in smears. Liver very large, nodular and fatty; no tubercle bacilli found in smears from crushed tissue. Spleen very large, nodular; one single acid-fast rod in smear from crushed tissue. Lungs, large isolated fibrous nodule. No tubercle bacilli found in smears from crushed tissue.

GUINEA-PIG No. 244. No treatment. Control.

Infected the same as guinea-pig No. 247. Weight 475 grams.

Died March 1, 1897. Weight 380 gms., a loss of 95 gms. in 138 days after infection.

Autopsy: Generalized caseating tuberculosis, with tubercle bacilli in all lesions.

GUINEA-PIG No. 246. No treatment. Control.

Infected the same as guinea-pig No. 247. Weight 635 grams. Died January 7, 1897. Weight 520 gms., a loss of 115 gms. in 86 days after infection.

Autopsy: Generalized caseating tuberculosis, with tubercle bacilli in all lesions.

* * * * *

GUINEA-PIG No. 254. Weight 685 Grams.

Treated August 21, 1896, to November 6, 1896, with 0.01 per cent solution of Watery Extract, in doses of 0.2 to 0.5 cc., a total of 57 cc., representing 5.7 mgr. of dried extract.

Infected November 11, 1896, with 0.01 mgr. of human tubercle bacilli; weight 765 gms., a gain of 80 gms. during treatment. No treatment after infection.

Killed September 7, 1897, or 300 days after infection. Weight 630 gms., a loss of 135 gms.

Autopsy: Infection-site not found. Regional lymph-glands enlarged, some with central caseation; no tubercle bacilli found in smears. Liver and spleen normal. Mesenteric glands caseous; tubercle bacilli found in smears. Lungs, few isolated fibrous nodules; degenerated tubercle bacilli found in smears from crushed tissue.

GUINEA-PIG No. 259. Weight 515 Grams.

Treated the same as guinea-pig No. 254.

Infected November 11, 1896, with 0.01 mgr. of tubercle bacilli subcutaneously. Weight 585 gms., a gain of 70 gms. during treatment.

Treated after infection, November 12, 1896, to March 3, 1897, with 58 doses of 0.01 per cent solution of Watery Extract, a total of 29 cc., representing 2.9 mgr. of dried extract.

Died October 22, 1897, or 345 days after infection. Weight 620 gms., a gain of 35 gms.

Autopsy: Infection-site not found. Regional lymph-glands fibrous. Liver and spleen enlarged. Lungs, numerous fibrous areas and more recent nodules; tubercle bacilli in smears from crushed tissues.

GUINEA-PIG No. 263. Control. No treatment.

Infected the same as guinea-pig No. 254. Weight 350 gms.

Died March 22, 1897. Weight 354 gms., a gain of 4 gms. in 131 days after infection.

Autopsy: Generalized tuberculosis. Smears show tubercle bacilli in all lesions.

GUINEA-PIG No. 265. Control. No treatment. Weight 510 Grams.

Infected the same as guinea-pig No. 254.

Died March 12, 1897. Weight 390 gms., a loss of 120 gms. in 121 days after infection.

Autopsy: Generalized tuberculosis. Tubercle bacilli found in all lesions.

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GUINEA-PIG No. 335.

Treated with 1 per cent solution of Watery Extract, from December 10, 1898, to February 8, 1899. Four doses, 0.25 cc., 0.5 cc., 1.0 cc., 1.0 cc., a total of 2.75 cc., representing 27.5 mgr. of dried extract.

Infected subcutaneously, March 2, 1899, with emulsion from caseous lymph-gland containing tubercle bacilli, obtained on autopsy of guinea-pig No. 271; weight, 560 gms. Died August 12, 1899, or 163 days after infection; weight 580 gms., a gain of 20 gms.

Autopsy: Infection-site thickened; regional lymph-glands, mesenteric and bronchial glands fibrocaseous; tubercle bacilli in smears. Organs normal.

GUINEA-PIG No. 336. Control. No treatment.

Infected March 2, 1899, the same as guinea-pig No. 335; weight 640 grams. Died May 17, 1899, or 47 days after infection; weight 510 gms., a loss of 130 gms.

Autopsy: Generalized tuberculosis; no record of smears.

* * * * *

While our older studies and experiments were not satisfactory and had left us in doubt, the advances made thereafter in re-

searches in immunity justified the resumption of our investigations, at first by studies in complement-fixation, which we conducted systematically for several years with sera from patients before, during and after treatment. In these studies it was found that the results often varied from day to day, and that the variations showed a relation to the administration of specific remedies, to exercise, to the titer of the opsonic index, and so on. In order to exclude possible technical errors, larger amounts of serum were taken and examined, and positive specimens were preserved and employed as controls, in which the titer was found to be constant.

The antibodies found being specific, their titer, when determined in quantitative tests, served us as an index of the specific resistance of the individual from whom the serum specimen had been taken.

In these studies we found patients whose sera, having been persistently negative as to complement-fixation before treatment, gave positive reactions on examination after comparatively short periods of treatment. It was then natural to inquire how many doses would be necessary to cause the maximal titer in the complement-fixation test, with our new preparation which we believed to be superior to others, including the Watery Extract of Tubercle Bacilli, in that it contained all bacillary constituents and yet was free from the danger of causing necrosis and abscess at the site of injection.

It did not require an excessive power of imagination to feel that we were approaching the realization of our aim of preventive vaccination more closely. It was a question of the number of doses, of their toleration without causing harm or unduly severe reactions, of uniformity in response, of simplicity in application and in the selection of suitable cases for its administration.

If these questions could be settled satisfactorily in infected children, we intended to recommend their vaccination on the ground that a greater resistance could be assumed to have been conferred on them because of the appearance of specific antibodies; if we could show like results in children who had as yet not been infected, their vaccination was justifiable for the same reason; and the prospect for additional support from animal experiments was good, since now we could test our treated

animals before infecting them, and could select our immune sera by the same test for the study of their bactericidal power, if this was at all demonstrable *in vitro*.

It should be borne in mind that we were working in two directions, one being the demonstration of protective substances in the form of specific antibodies, the other the improvement of the specific antigen or vaccine which we proposed to administer for the production of these antibodies. The results of these studies and experiments formed the basis of our first report of June 1, 1912.

The increasing conviction that the whole bacillary substance was necessary, the generally observed liability to the production of necrosis and abscess with the use of tubercle bacillus emulsion, unless the doses were small and the increase was gradual, our unwillingness to employ a living virus in the human subject, and the impracticability of the intravenous mode of administration for general application, all these considerations had caused us to direct our efforts toward the improvement of the Watery Extract of Tubercle Bacilli which, as we then understood it and believed, contained only the water-soluble proteins of the virus and was therefore not a complete antigen.

We had approached the problem of improving our Watery Extract of Tubercle Bacilli in the spring of 1906, first by chemical studies of the tubercle bacillus, and thereafter by producing numerous preparations in which the water-soluble proteins were supplemented by other constituents of the tubercle bacillus, which were less soluble, and by small amounts of the bacillary fats. These preparations were, in 1907, first tried upon animals, in order to determine their toxic and necrosing action when administered subcutaneously; finding no cause for objection in this respect, we then tried one of them clinically in tuberculous patients, apparently with such gratifying results as to induce us to place this preparation at the disposal of several clinicians, in order to check up our own observations.

Our attention had been directed to the importance of the fats contained in the tubercle bacilli, for purposes of immunization, by the experiences of Deycke in lepra. Deycke Pasha & Reschad Bey [20] had isolated a neutral fat from cultures of the Streptothrix leproides, which they called *Nastin* and which they found to

[20] Deycke Pasha & Reschad Bey; *loc. cit.* (p. 118).

be an essential factor in the specific treatment of leprosy. It was of further importance that Bang & Forssman [21] could show that the lipoid substances of red blood-cells give rise to the production of specific hemolysins; and finally other authors found that complement-binding substances could be produced by treatment with certain lipoids.

It has been denied that pure lipoids possess antigenic properties, and the development of antibodies, after immunization with lipoids, has been attributed to the admixture of protein-substances. Deycke & Much [22] have demonstrated an immunizing effect with a mixture of nastin and of bacillary proteins in solution, which could not be obtained with either the lipoid or the protein alone.

In January, 1908, we employed deliberately, as the first, A WHOLE VACCINE, IN SOLUTION, OF THE TUBERCLE BACILLUS for clinical purposes, which contained, in addition to the soluble proteins, one milligram of the tubercle bacillus-fats per cubic centimeter. We were therefore not only the first to employ, for immunization, the proteins of the tubercle bacillus in solution, which we did in 1897, but we were also the first to act upon the recognition of the fact that ALL ACTIVE SUBSTANCES OF THE TUBERCLE BACILLUS, INCLUDING THE BACILLARY FATS, ARE REQUIRED FOR A COMPLETE IMMUNIZING EFFECT, and the first to put this principle into practise by the clinical administration of a complete antigen in solution.

Dr. H. L. Taylor of St. Paul, Minnesota, was one of the clinicians selected by us to undertake clinical studies with the preparation in question, and he referred to his experiences with it briefly in the first paragraph of a paper [23] in which he published the results of his observations with our present vaccine.

We were obliged to discontinue the use of this preparation because necrosis and abscess followed its administration at the point of injection, although it contained only one milligram per cubic centimeter of the fatty extracts, while our analysis of the whole substance of tubercle bacilli had shown them to contain from twenty-five to forty per cent in fats of their total dry weight.

[21] BANG & FORSSMAN; Centrlbl. f. Bakt. Orig. 1906, XL, 151.
[22] DEYCKE & MUCH; *loc. cit.* (p. 118).
[23] H. LONGSTREET TAYLOR; St. Paul Medical Journal; 1914, July.

The abscesses which were then observed did not appear as promptly as one would expect ordinarily; the first one formed six weeks after the injection of a small therapeutic dose. This and subsequent injections had given rise to local reactions consisting of areas of circumscribed redness, swelling, heat, and tenderness to pressure, but apparently not differing from like local reactions which we had been in the habit of noting in our early experiences with similar preparations, including our Watery Extract. The reactions subsided as usual and, during the six weeks since giving the first dose, we had administered six more, when it was noticed that the injection-site of the first dose again appeared slightly swollen and red and that it was painful.

The marked and striking clinical improvement in this case (a child with an open destructive lesion in one lung) had before this time become so convincing that we had started treating a number of other patients with this vaccine, and we were considering a still more extensive clinical use of it, when the observation in the child's case was made. In further respect to it, it is only necessary to say that sterile abscess developed a few days later, that like abscesses occurred successively at the sites of all previous injections, and that our experience was the same in all patients who had been given one or more doses of this preparation. Although reluctant to give up the clinical use of this vaccine, as was also Dr. Taylor, who was so pleased with it that he desired a further supply and was ready to take the disadvantages of abscess-formation into the bargain, we made no more of it. A new preparation containing only one-tenth milligram of fat-extractives, which we tried a year later, still caused abscess in doses of 0.05 cc., although not so uniformly, and these accidents were never eliminated completely until the fat-content of the vaccine was reduced to the amount in which it appears in our present formula.

When we were satisfied that the necrosing effect was overcome, and we had no longer need to fear abscess, we studied the effect of our vaccine upon the production of antibodies as demonstrated by laboratory methods. We had already learned that serum-specimens taken from the same individual on successive days were sometimes low, sometimes high in their antibody-contents, that the negative and positive phases in tuberculous sub-

jects were likely to alternate, and that our doses were always followed by a negative phase, exactly as Sir Almroth E. Wright had shown to occur after the injection of tubercle bacillus emulsion. The difference in our cases was that the negative phase was not prolonged over weeks, as he observed it, but that it gave place to a positive curve in the course of a few days.

The reason for this difference is not difficult of appreciation on considering that we worked with a vaccine in solution which could be administered in comparatively large doses, and the prompt absorption of which was followed by an equally prompt reaction, whereas Wright was limited to the use of minute doses of an emulsion of tubercle bacilli which had to be dissolved and fitted for absorption in the living body by processes so tedious and uncertain that a prompt response could not be expected.

We concluded, therefore, that a vaccine which is suitable for practical prophylactic immunization must not only contain all bacillary constituents, but that these must be in solution and that the preparation must be so balanced in its fat-content as to eliminate the danger of necrosis or abscess, the occurrence of which would preclude its general use, even if it were otherwise efficient, because the interested public would refuse it or, at all events, would object and, from its standpoint, perhaps properly. The possibility of ulceration at the site of vaccination is a potent cause of the fear of prophylactic vaccination against smallpox, and the justice of this fear cannot be denied on contemplating the shocking disfigurements which are sometimes seen on the arms of patients in the consultation-room. Physicians themselves will appreciate this on reflecting what it would mean for parents to have to accept an ugly, slowly healing sore upon the body of a loved baby or child, the need or the value of whose protection against tuberculosis they could, as a rule, not appreciate at the time, and, vaccination having been neglected, not until after the signs of clinically manifest disease had declared themselves.

Numerous serum-specimens from individual patients were again examined daily, in order to determine the time of appearance of antibodies after a dose of vaccine had been administered, and a critical examination of the charted results caused us to select the fifth day as the proper one for repeating the dose,

because at that period the low point of the negative phase had usually been passed and the positive phase was again present.

We are not prepared to show that the administration of vaccine during the negative phase would be detrimental, and we are well aware of the practise of many clinicians of giving small doses of Tuberculin or of Bacillus Emulsion, even daily or at short intervals, without any serum-control whatever; in fact, we have done so ourselves in the past. We feel, however, that there is much to be said in favor of a mode of administration by which a continued negative phase is guarded against and the organism is given a chance for reaction, especially when the doses are large. We are also conscious of the fact that the interval of five days is more or less arbitrary and that, at least theoretically, serum-tests are always necessary for absolutely exact work. We found, however, by numerous daily tests on many patients that, in a given case, a dose on the fifth day or five days after the subsidence of a general, febrile reaction as a rule coincided with the reestablishment of the positive phase, the serum-titer being then at its acme or near it and, finding moreover by control examinations that, by the time the result of the serum examination could be known, the titer was apt to have changed again, we accepted our general results as sufficiently exact for practical purposes, and we have given our doses on this plan since then.

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In our first report on a practical method of prophylactic vaccination against tuberculosis,[24] it was pointed out that the object of the studies leading up to the development of this method rested in the accomplishment of a practical means for protective immunization against tuberculosis, and that the proposed method must be safe and simple, and its results must be uniform, if it was to prove serviceable for general application, the same as is the case in vaccination against smallpox or against typhoid fever. As our additional experiences have shown that our method meets these requirements, it is our purpose to lay the accumulated

[24] KARL V. RUCK; Journ. A. M. A.; 1912, LVIII, 1504.—A Practical Method of Prophylactic Immunization against Tuberculosis with Special Reference to its Application in Children; Pamphlet, Asheville, N. C., 1912. —Medical Record; 1912, LXXXII, 369.

evidence before those who are interested in the procedure, with a view to its general adoption.

In the publications referred to we fully described the vaccine which we employ, giving a detailed account of its manufacture, and we did not fail, at that time, to point out the necessity of continuing the related experiments, and the need of further practical studies. Concerning the vaccine, as then prepared, we disclaimed entertaining the idea that it alone could have produced the results which we had observed, but we expressed the belief that a preparation intended for prophylactic use, especially in a single dose, not only must contain all constituents of the tubercle bacillus, but that these should be properly proportioned and in solution. These conditions are complied with in our vaccine.

Since then certain experiences obliged us to make some changes in the technical details in the manufacture of the vaccine for the purpose of preventing deterioration of its constituents, which had been discovered in the fall of 1913,[25] and of securing the absolute uniformity of its action. Requiring both chemical and biological investigations, these studies were necessarily tedious and are not yet completed entirely; but they have yielded enough information at this time so that the occurrence of chemically demonstrable changes, with the increasing age of the vaccine, is now prevented.

* * * * *

Prior to our writing in 1912, the subject of vaccination had been proposed by several authors, especially by Heubner, v. Behring, Calmette, and by others, without any practical application of such a method having been undertaken. Petruschky has insisted for many years upon the importance of prophylactic immunization, especially of children living in families in which there are tuberculous members, and he attempted to secure it by means of persistent periodical courses of treatment with tuberculin. In order to avoid the many disadvantages of subcutaneous injections, he finally developed C. Spengler's method of percutaneous administration, for which he now employs a "liniment" containing a glycerin-extract of dried human and bovine tubercle bacilli, a small amount of which is applied on

[25] KARL v. RUCK; Experiences in Prophylactic and Therapeutic Immunization against Tuberculosis. Pamphlet, Asheville, N. C., 1914, p. 7.

alternating places of the body, at intervals of three days. The applications are made by the persons to be immunized, on their own bodies.

In Hela, a peninsula projecting into Danzig Bay on the Baltic Sea, which is inhabited by fishermen and is practically isolated from the world, Petruschky [26] instituted, in 1911, the deliberate "assanation" of all families in which there were tuberculous persons, or individuals who reacted to the tuberculin test. In addition to the very encouraging therapeutic results which followed, he succeeded in restoring the "pretuberculous" persons, mostly children, to health, and could report recently that the inhabitants of Hela are now entirely free from tuberculosis.

Petruschky's method was employed also by Kutschera [27] in several nuns' convents in the Tyrol, which were permeated with tuberculosis so seriously that all novices acquired the disease within one year of their admission. Kutschera reported excellent results after an observation of only nine months. He emphasizes the fact that the development of open tuberculosis can be prevented by timely specific treatment and that, in this manner, the development of future sources of contact-infection is avoided.

Petruschky's experiments appear to have shown that whole tubercle bacilli can be made to be absorbed by the skin, and that they are broken up in the process and disintegrated. He decided upon the glycerin-extract, after employing various extractives in his endeavor to obtain a whole antigen, and especially to secure the lipoid constituents of the tubercle bacillus, which are supposed to be essential for immunization. The disadvantages of his method lie chiefly in the necessity of applying his preparation by the percutaneous route, which makes it slow and tedious and more or less uncertain in results, becoming in fact a course of prolonged treatment. In the light of our own experiences we doubt that absorption and disintegration of tubercle bacilli occur through the skin, and, inasmuch as Petruschky's results were obtained in infected individuals, we believe that they can be explained satisfactorily

[26] J. PETRUSCHKY; Beitr. z. Klin. d. Tuberkulose; 1914, XXX, 217.—Münch. med. Woch.; 1915, LXII, 145.

[27] AD. KUTSCHERA; Münch. med. Woch.; 1914, LXI, 974.—Wien. klin. Woch.; 1914, XXVII, 799.—Tuberculosis; 1914, XIII, 381.

through the action of the substances which were contained in the glycerin-extract which in reality corresponds with tuberculin and therefore stimulates the development of spontaneous or auto-immunization through the occurrence of focal reactions in the tuberculous organism.

In an interesting recent communication, Dr. W. M. Crofton,[28] of Dublin, relates that it has been his practise for the last eight years to urge the members of families whose parents were tuberculous or in which cases of tuberculosis had occurred, to undergo short courses of treatment with tuberculin. Under the use of Tuberculin TR for prophylactic purposes, the improvement in health, in many of the members of tuberculous families, has been most satisfactory and, so far, no case of tuberculosis has occurred among those so treated; but as the author points out himself, the numbers are far too small to have as yet any value.

Realizing the deficiencies, as antigens, of Tuberculin TR and of all other tuberculins on the market, Crofton has prepared what he believes to be a whole antigen for therapeutic and prophylactic immunization by dissolving tubercle bacilli in benzoyl chlorid solution, diluting the solution with liquid paraffin, to represent two per cent of the benzoyl chlorid solution, and adding iodoform dissolved in ether "so as to combine immuno- and chemo-therapy."

In Crofton's opinion, "there is only one method of bringing up a low resistance to normal or of increasing a normal resistance, and that is the production of specific antibodies in the individual, by inoculating him with a vaccine of the related microbe. In the case of a universal infection like tuberculosis, it is the only measure that will protect when the constitutional resistance is lowered by the various environmental and inherited causes of low resistance."

He believes that, "while everyone ought to be vaccinated against tuberculosis, so as to make sure their resistance is at least normal, a beginning might be made with members of families in which tuberculosis has occurred, since their resistance is usually subnormal and they have peculiar opportunities for contracting the disease." This is exactly what we proposed four years ago and on many occasions since then.

[28] W. M. Crofton; Brit. Med. Journ; 1915, I, 629.

Reference may also be made in this connection to a report by Helwes [29] of an attempt to prevent the further development of open cases of tuberculosis in an entire country district by treating all closed cases with tuberculin; Old Tuberculin, Bacillus Emulsion and Albumose-free Tuberculin were employed for the purpose. The undertaking was carried out under the auspices of a county tuberculosis commission and had a twofold effect; first, the restoration of many closed cases of tuberculosis to health, thus preventing their becoming infectious, and second, that of educating the very conservative people of this particular country district to consult the physician in good time, and to take advantage of the general methods employed for the limitation of tuberculous disease.

We have repeatedly referred to the slow and incomplete spontaneous development of specific immunity against the tubercle bacillus at a sufficiently early period of infection, and have explained it by the lack of solubility of its body-substances. It was this lack which caused us, twenty years ago, to undertake their artificial extraction in the preparation of a purified tuberculin and, thereafter, of a watery extract made from the bodies of the bacilli; it likewise governed us in the preparation of our vaccine. The resistance of tubercle bacilli to ordinary solvents, as well as in tuberculous tissues, has long been known and is generally recognized.

Petruschky [30] accounts for the difference in rapidity and degree of the spontaneous development of immunity, between certain acute diseases and tuberculosis, by citing the facts that tubercle bacilli are provided with an impermeable fatty-waxy capsule, that they are of very slow growth, and resist destructive influences, and by the peculiar behavior of the body in response to their invasion. The attempt of the organism to isolate the invading bacilli by encapsulation leads to the characteristic phenomenon of tubercle-formation. This same encapsulation prevents any considerable transition of specific substances of the tubercle bacillus into the body-fluids and thereby hinders the stimulation of a marked antigen-effect as it is observed to

[29] HELWES; Zeitschr. f. Tuberkulose. 1913, XX, 32.

[30] J. PETRUSCHKY; Grundriss der spezifischen Diagnostik und Therapie der Tuberkulose. Leipzig, 1913, S. 47.

occur spontaneously in most acute bacterial diseases. While the point of infection is overloaded with specific poisons, and for this reason succumbs more or less to necrosis, too few of the bacterial poisons get into the body at large to train and stimulate the defensive apparatus sufficiently. As soon as the encapsulation is broken through, e.g., when a tuberculous focus with necrotic center breaks down, not only specific poisons but also living tubercle bacilli enter the body-fluids and may lead to metastases, to new colonizations of tubercle bacilli with, again, encapsulation or tubercle-formation. Thus the vicious circle is established which renders the activation of natural healing processes very difficult and often futile, because they are insufficient.

"If, however, specific bacillary substances are introduced deliberately into the body, the antigens can produce the necessary reactions and the organism is thus enabled to form antibodies without the associated risk of colonization of new tubercle bacilli, that is to say, of an extension of the disease. The antigen-action being specific for the part as well as for the whole, the antibodies are formed for the several constituents of the tubercle bacillus of which the antigen consists, which illustrates the necessity of employing a whole antigen for effective immunization.

"By the introduction of specific remedies, therefore, the natural healing process, as it is observed to occur in bacillary diseases, is promoted, and it becomes possible to heal tuberculous disease in a natural manner, and also to prevent it in the same manner as it is prevented spontaneously, in instances in which the infection never develops into actual disease."

As we have considered and investigated the subject of prophylactic vaccination against tuberculosis only from a thoroughly practical standpoint, as applicable by the general practitioner, and with a view to its adoption for the benefit of the masses, it has for us not been a question whether a specific immunity could be produced at all against the tubercle bacillus and whether, if produced, it would prove of practical benefit; on these points we have satisfied ourselves years ago in the affirmative sense. The object of our studies has been to find a practical method, free from all objections in the sense referred to, a method which might be recommended for use in all children who had suffered exposure to infection in their homes, and eventu-

ally for use in all other children as well as in all adults. AFTER NEARLY FIVE YEARS OF PRACTICAL EXPERIENCE, WE ARE STILL CONVINCED THAT A WHOLE VACCINE IN SOLUTION, WITH NO GREATER AMOUNT OF THE BACILLARY FATS THAN OUR VACCINE CONTAINS, WILL MEET ALL REQUIREMENTS OF SAFETY AND SIMPLICITY OF ADMINISTRATION, AND ALSO ALL REQUIREMENTS OF EFFICIENCY AND OF UNIFORMITY IN THE PRACTICAL RESULTS.

GENERAL CONSIDERATIONS OF SPECIFIC PROPHYLAXIS

Since the publication of our first report, our method of prophylactic vaccination has been applied further, by ourselves, in 1512 children and in 106 adults. Dr. Julian [31] of Thomasville, North Carolina, has vaccinated over 200 additional children in the Baptist Orphanage of that place, and numerous physicians elsewhere have used the vaccine for like purposes. Several of these independent observers have published the results of their experiences which, like our own, were chiefly with children who were or had been exposed to infection in the family and in whom clinical manifestations of tuberculous disease were already in evidence. Dr. Flack,[32] formerly of Spray, North Carolina, now Professor of Pathology in Wake Forest College, made extensive use of the vaccine in connection with the Welfare Work in the families of factory employees, using it for prophylactic and for therapeutic purposes in over 500 cases. Dr. Frank Neall Robinson [33] of Monrovia, California, made a preliminary report on thirteen cases. Dr. J. J. Terrill [34] of Temple, Texas, reported on his results in eleven cases. Dr. W. H. Watterson [35] of Waukegan, Illinois, published his results in ten prophylactic cases, and in 31 early, 33 moderately advanced, and 15 far advanced cases of tuberculous disease, including two cases with laryngeal tuberculosis. Certain results observed by Dr. B. F. Terry [36] of Rising Star, Texas, who has probably

[31] C. A. JULIAN; Medical Record; 1913, LXXXIII, 1059.

[32] R. E. FLACK; Charlotte Medical Journal; 1914, June, and 1915, February.

[33] F. N. ROBINSON; Calif. State Journ. of Medicine; 1914, March.

[34] J. J. TERRILL; Texas State Journ. of Medicine; 1914, December.

[35] W. H. WATTERSON; Illinois Medical Journal; 1915, February.

[36] B. F. TERRY; New Orleans Med. and Surg. Journal; 1915, LXVIII, 81.

the largest individual experience in the application of the vaccine, were recently published. Dr. Taylor [37] reported, among others, on the cases of two children vaccinated by him in an orphanage, which are of particular interest because the results are attested by the physician in charge of the institution, and also because the relation of the vaccination to the results is so evident that no room is left for doubt. Mention may be made again of the first publication on the practical application of the vaccine, by Dr. Julian, in which the information given in our first communication on the subject was presented in greater detail than it had been possible for us to do. It may be mentioned further, in this connection, that in response to an inquiry [38] among other physicians, in regard to their experiences with the Watery Extract of Tubercle Bacilli and the Vaccine, fifty physicians report good results from the prophylactic use of the Vaccine, while two physicians report negative results.[39] Of the former, forty observed a marked increase in weight in their vaccinated children; three failed to note such an increase. From the prophylactic use of the Watery Extract, eleven physicians report good results, and seven of them have observed an increase in weight.

The object of our additional studies was chiefly to extend our observations to a sufficiently large number of cases which were to be controlled by biological studies and by bactericidal experiments upon animals, with serum taken before and after vaccination. The bactericidal experiments, finished and reported upon in 1912, were only sixteen in number, and it was desirable to ascertain with what degree of uniformity like results could be obtained in a larger series of experiments. It was further advisable to determine whether, in case the animals were not killed or at any rate were allowed to live much longer than the

[37] H. L. Taylor; *loc. cit.* (p. 129).

[38] K. v. Ruck & Silvio v. Ruck; A Clinical Study of 965 Cases of Pulmonary Tuberculosis. Pamphlet, Asheville, N. C., 1915, p. 20, ff.

[39] In normal, healthy children, the results of prophylactic vaccination against tuberculosis can necessarily not become apparent in a general improvement in health, since this had not been impaired; in these children the vaccination is undertaken purely as a measure of precaution, like vaccination against smallpox. The same holds good in the case of infected or tuberculous individuals who are as yet free from symptoms and whose nutrition has not deteriorated on account of a tuberculous toxemia.

controls, the infection would not develop at later periods; and finally we wished to know for how long after vaccination the bactericidal power of immune serum could be demonstrated; in other words, how long the artificial immunity could be proved to persist by laboratory tests.

In our report of 1912 we mentioned the results of our first reexaminations which were made six months after vaccination in 118 children at the Thomasville Orphanage, who had presented physical signs of structural changes in the lungs before they were vaccinated. At that time we spoke only of improvement, because it was possible that the results then found might not be permanent. Later reexaminations, the results of which were reported by Dr. Julian, the physician in charge, have shown, however, that no relapses have been observed. In this connection we refer with considerable gratification to a personal communication from the Rev. Dr. Kesler, the superintendent of the institution, to the effect that, up to January, 1916, none of the vaccinated children had lost the improvement following upon their vaccination, and that they continue in good health. In two cases of open tuberculosis, treated with eight slowly increasing doses of vaccine, a relapse was noted, however, during the past year: in one case after a severe attack of pneumonia; in the other after persistent indiscretions. A third case also failed to maintain the improvement which had been evident before.

The uniform general improvement and the disappearance of signs of tuberculosis-infection in the vaccinated and treated children, were so unmistakable and decided that, even if we had been unable to support the results of Dr. Julian's and our own clinical examinations by confirmatory laboratory evidence, the administration of the vaccine in similar cases would not only be justified, but urgently demanded as a practical therapeutic measure and as one that may be expected to prevent the subsequent development of phthisis after infection has taken place. One or two doses of vaccine having been sufficient to produce these results, and their administration constituting, in addition, a diagnostic test as reliable as any other and more so than the cutaneous tuberculin test, no good reason whatever exists why the vaccine should not be administered to children in whom we have reason to suspect an infection with tubercle bacilli. In

such cases it is a logical procedure, the same as is a tuberculin test; but, unlike the latter, it has the important advantage that, in the tuberculous, its substitution for tuberculin is followed by improvement and by the restoration of the general health when this has been impaired by reason of an existing infection. The clinical observer can demonstrate such a result in every case, without laboratory tests and without recourse to experiments, by the administration of the vaccine.

In our clinical observations only one seeming contradiction has been noted, in a boy ten years old, whose record showed previous and continued exposure to infection. Examination disclosed a sallow complexion, irregular rises of temperature, enlarged lymph-glands, slight physical changes in one lung, deficiency in weight and strength. The boy reacted locally and generally to a small dose of vaccine but, although he received additional doses and finally the maximal dose, there was not only no general improvement but he lost several pounds in weight and the fever failed to disappear.

The boy, who lived at a distance, was permitted to return home with the understanding that he should be brought back for further examination if he did not improve. When this was done a month later, our examination showed decided improvement of physical signs in the lung, but the fever had continued and he had lost more weight. The charted temperature records caused us to suspect a complicating malaria; plasmodia were found in the blood, and the patient slowly improved under antimalarial treatment which we instituted and which was continued at home until his recovery was established.

CHAPTER VI

THE ADMINISTRATION OF THE VACCINE

SELECTION OF CASES FOR PROPHYLACTIC VACCINATION

Examination of Patients before Vaccination

We may perhaps aid some practitioners in the selection of cases for the prophylactic and the therapeutic use of the vaccine respectively, by pointing out the considerations which govern us in our practical work, and by discussing them critically.

Before deciding whether a particular individual, especially a child, is suitable for the administration of the vaccine in doses given for prophylactic purposes, it is well to ascertain whether there has or has not been a known exposure to infection, especially in the family. In the case of children in families in which there is a tuberculous patient, an infection is the more likely to have taken place the younger the children are and the more intimately they have come in contact with the person or persons affected with open tuberculosis.

For the purpose of physical examination, it is necessary to remove the clothing so as to give access to the entire body down to the hips, covering the child with a loose wrapper for protection. The first part of the examination is to make a general survey of the child's appearance and state of nutrition. If an exposure to infection is likely to have occurred in the family, we are not surprised if the child appears less robust and if his appearance is more delicate than would be expected in a normal child of the same age, or if his nutrition, as measured by weight, is lacking more or less. With respect to the state of nutrition, we are, however, not governed entirely by the number of pounds the child weighs, but take into consideration also the body-length, as well as the size and massiveness of the bony frame. Next we note the condition of the skin, its color, smoothness and elas-

ticity, and that of the conjunctival and oral mucous membranes, incidentally also investigating the condition of the teeth, gums, tonsils and of the pharynx. We further note the upper arms, to see whether they are round and full, or whether they are rather thin, as is often the case in tuberculous individuals. All this is a matter of a minute or two, taking practically no time at all; the trained eye can take in all these points at a glance.

Having looked over the child in a general way, the inspection of the chest follows, and this is important, even in very young children. Noting its general form and contour very carefully, we watch the respiratory movements, especially in the upper portion of the chest, in order to determine whether the expansion is good and equal on both sides, and we look for lagging or for circumscribed areas of slight or of absent motion and even for retractions especially above and below the clavicles, bearing in mind whatever appears suspicious, for particular attention on percussion and auscultation. The former we practise at first very gently, trying out rather for slighter degrees of shortness of the percussion-note than for markedly dull or flat sounds.

If gentle percussion shows no changes, forcible percussion will rarely bring them out, but comparing both sides, we should not forget that bilateral lesions may be present in an early stage, even in children; in any case, we must remember that the percussion-note is normally slightly shorter and a little higher in pitch on both sides immediately below the sterno-clavicular junction. In our experience with children, we found that early unilateral signs occur in the upper lobes with equal frequency as we find them in adults, and also that they have a tendency to extend downward; we therefore pay particular attention to the upper third of the chest, both anteriorly and posteriorly, and we carefully watch for percussion-changes between the scapulæ, on account of the possible enlargement of the bronchial and hilus-glands. When these are suspected, we try for tenderness on pressure upon the spinous processes of the corresponding vertebræ, and resort to x-ray examination when this is deemed necessary.

In auscultating for early phenomena we do not expect to find bronchial respiration, or râles, or rhonchi often; these signs belo to older lesions or to active destructive disease if it is tubercul Bearing in mind what we had found to be appar

on inspection and percussion, we expect to discover in the suspected localities more or less loss of the vesicular (or puerile) quality, which is replaced by a harsher, sharper character of inspiratory sounds, with or without continuous and prolonged expiration, and if we find this to correspond to a circumscribed area which we have already under suspicion, because of what we saw on inspection or heard on percussion, or both, we are reasonably certain that this area is the seat of pathological changes, even though we were to hear no râles or other coarse sounds. Such areas may show in addition an increased vocal fremitus, and the auscultatory signs may become intensified when the child takes a deep inspiration or is made to cough.

Our examination of the chest being finished and the results recorded, we proceed to that of the lymph-nodes in the neck, axillæ and groins. On examining the cervicals, we include the posterior group and, in order to find also small nodes, we palpate gently and with a sort of rolling or oscillating motion of the finger tips; by inclining the head laterally and again inward and forward, we get a better chance to feel the enlarged nodes than when the muscles are tense and, on palpating behind the clavicle, bending forward the child's head permits the fingers to be insinuated more deeply when we stand or sit behind rather than in front. The axillary lymph-nodes are enlarged more often than is usually supposed; they are very movable and some of them are situated high up in the apex; gentle palpation of the entire axilla and a rolling or stroking motion by which we bring a gland upon the firm base of the rib, lead to the detection of even very small nodes. The inguinal glands are found readily if they are enlarged; but here also the partial flexion of the thigh upon the abdomen may aid in their detection.

Having completed our records of the several groups of lymph-nodes, we make necessary additional inquiries concerning the child's appetite and digestive functions, the frequency of catarrhal affections of the air passages, and, unless we already have a temperature record with three or four observations for at least the preceding day, we arrange for such measurements to be made. On the next or the following day we are ready to vaccinate the child with a single full, or with a smaller trial-

(diagnostic) dose, as we may think best from the results of our examination.

We interpret the results of the probable findings in the order in which we have described the course of physical examination, as we are in the habit of making it, as follows:

1. The Clinical History.—If this is positive, the history of a known exposure to infection in the family having been elicited, we have good reason to anticipate that we shall obtain other confirmatory evidence; whereas if it is negative, the probability of finding evidences of an infection becomes the less the younger the child is.

2. The State of Nutrition.—Before we accept any apparent deficiency in weight or in the roundness of form, in color and appearance of the skin and mucous membranes as having a tuberculous basis, we seek to eliminate other possible factors that may stand in causative relation, these being chiefly disturbances of the digestive functions originating in, and more or less perpetuated by, unsuitable or improper diet and indulgences, lack of attention to proper evacuations, etc. In addition to this, we eliminate the sequelæ of preceding diseases such as acute catarrhal affections, measles, scarlet fever, rickets, etc., which may have left functional disturbances or organic lesions, and we must further think of malaria, hookworm, and of an inherited syphilitic taint, all of which may account for deficiencies that we may have found. No other reasonable cause being discoverable, the nutritive deficiency alone justifies us in suspecting tuberculosis, and if it is found in a child that is known to have been exposed to infection, the probability is very strong indeed that the deficient state of nutrition stands in relation to tuberculosis.

3. Physical Signs in the Lungs.—If the chest examination is negative, the fact alone does not in any way eliminate a tuberculosis-infection and it does so the less, the younger the child is. The infection need as yet not have involved the lungs. A negative finding may also be due to the fact that the lesions are situated deeply or that any tubercles which are present have not yet caused structural alterations sufficiently large to be detected. If we find physical signs, we must eliminate the sequelæ of preceding inflammatory changes, especially such as may follow upon pneumonia or even upon acute bronchitis involving the capillary

bronchi. Physical signs due to other causes than tubercle are comparatively rare, they do not persist and are not so circumscribed; yet the fact that another origin is possible must not be overlooked if the child has recently suffered from broncho-pneumonia or bronchitis. On the other hand, it is well to remember that a broncho-pneumonia is often of tuberculous origin. If its course has been irregular and its onset without a preceding catarrhal affection of the upper air passages and bronchi, if physical examinations during its acute course have shown the accompanying bronchitis to be limited to one side or to one lobe, if convalescence and recovery have been tedious or incomplete, the tuberculous nature of persisting signs is more than probable. If we find retractions over which the percussion-note is short, and the respiratory murmur not normally vesicular, we may be practically certain that tuberculous changes stand in relation, but we may also look upon the retraction as a sign of a healing or healed process for the lesion in its particular locality.

4. Enlarged Lymph-nodes.—The submaxillary group and, to a lesser degree, the upper cervicals become frequently enlarged from skin affections of the head and scalp, from diseased teeth and in connection with other oral affections including those of the faucial tonsils, also with catarrhal affections of the ear and the nasal and pharyngeal mucous membranes; but they may return to a non-palpable state almost as rapidly as they become enlarged after the cause has disappeared. It is usually possible to eliminate the presence of these affections; but we must still think of pseudo-leukemia, Still's disease and other glandular diseases as possible factors, especially when large packets of glands are found. In the absence of other causes, our suspicion of a tuberculous origin increases as we find the enlarged glands in descending clusters and chains, especially in the deep and posterior cervical groups. In considering the axillary and inguinal groups, we must eliminate acute and chronic skin affections of corresponding parts of the trunk and of the extremities, and, in the inguinal groups, also catarrhal inflammations of the genitals. When, after all these considerations, we can eliminate other causes and have also remembered that several causes may coexist in one or the other groups, and if then we are still at a loss to account otherwise for the presence of enlarged lymph-nodes, we

are justified in considering them as probably of tuberculous origin.

5. Fever.—Marked fever is rarely present in tuberculosis unless there is progressive formation of tubercles in internal organs, or unless tubercles have caseated and the caseation is attended by softening of the focus. When tubercles are forming and extending but slowly from a prior localization, the temperature is elevated only slightly if it is influenced at all, and this is true when absorption occurs from only one small caseous lesion; but it is then apt to be increased by exercise. No inferences are justified from the occurrence of slight elevations of temperature on a single day, and in such a case it is well to continue the observations for a week or longer before coming to a decision, during which time other probable causes of the fever may be considered and removed.

If no other cause is found for existing temperature rises, and if elevations of one or more degrees persist, the probable relation of tuberculosis must be supported further, if possible, by repeated physical examinations, especially of the lungs to which an extension of the tuberculous processes is always possible. If pulmonary caseous foci stand in relation, the temperature will probably reach 100° F. and over; the physical signs are then apt to be more marked over the related focus, and the activity will manifest itself sooner or later by the appearance of catarrhal signs over the involved area, at least in the form of crepitation on deep inspiration and after coughing.

THE PROPHYLACTIC ADMINISTRATION OF THE VACCINE

Since our first report was published, we have had no occasion to modify the mode of administration of the vaccine materially. We have, however, been obliged to reduce the percentage of proteins in the preparation from one to one-half, having found that they remain in solution more certainly in a lower concentration, and in consequence the dosage, which was recommended at first, had to be increased. In a pamphlet published three years ago,[1]

[1] Karl v. Ruck; Some Practical Points in the Use of My New Vaccine and of the Watery Extract of Tubercle Bacilli. Pamphlet, Asheville, N. C., 1913.

this subject was considered further, and the doses given below were recommended, according to the age of the individual to be vaccinated.

The Subcutaneous Administration of the Vaccine.—In cases in which two or more doses are to be given, it is best to avoid injecting several doses in the same place. This precaution is perhaps less important in cases of a single repetition than it is in the repeated administration of the vaccine for therapeutic purposes, and the reader is referred to p. 262, where we have given our observations relating to the subject, and discussed the reasons for this precaution.

The full, single and maximal dose of the undiluted vaccine, given subcutaneously, is as follows, for the several age periods:

0 to 1 years	0.1 cc. to 0.2 cc.
1 to 6 years	0.2 cc. to 0.4 cc.
6 to 12 years	0.4 cc. to 0.6 cc.
12 years to adult age	0.6 cc. to 1.0 cc.

In the adjustment of the individual doses, intervening age periods, and the state of nutrition, as shown by the body-weight and by the general appearance, are to be taken into consideration.

We have found that these doses are ample and, as a rule, larger than necessary for a complete immunizing response, resulting in a maximal amount of specific amboceptors for tubercle bacilli and for all their partial antigens, and in the development of a complete bactericidal power of the serum.

In numerous instances we tested the sera for their amboceptor-contents and their bactericidal power, after a general reaction had occurred to less than the full dose, and found the results satisfactory, which suggests that, whenever a general reaction follows the administration of a dose of vaccine, a sufficient amount of bactericidal antibodies may be assumed to have been produced, regardless of the size of the dose which had caused the reaction. We are continuing our observations on this point and anticipate that in course of time we may be justified in reducing the doses, as they are tabulated here, materially.

The Intravenous Administration of the Vaccine.—We have resorted only infrequently to the intravenous administration of the vaccine and, as far as we have observed them, the effects

are identical with those that are found to follow after the subcutaneous injections; with this difference, that reactions appear more promptly and that the dose or doses may be materially smaller. We have given only one-half of the subcutaneous doses.

We have, however, a larger experience with the intravenous injection of vaccine in the immunization of animals. While we did not find the technic easy in guinea-pigs, and often lost these small animals when the vaccine had been injected directly into the ventricles of the heart, we succeeded very well in rabbits, in which we finally produced a complete immunity by means of a single dose equivalent to ten cubic centimeters of the vaccine.

In deciding whether the vaccination shall be made with a single or with several doses, we have been governed by the following considerations:

(1) We give the full subcutaneous dose for the particular age period:

(a) To children who, although exposed to infection, have not suffered seriously in nutrition; who may or may not have enlarged lymph-glands, but present no physical signs in the lungs; whose temperature has, during the preceding day, been normal or has not exceeded 99° Fahrenheit.

(b) To children complying with the foregoing conditions, who have slight signs in their lungs, but whose temperature has been strictly normal.

(2) We give a small beginning dose in all other cases where the temperature does not exceed 100° Fahrenheit.

(3) We delay the administration of the vaccine in all cases in which the temperature record for the preceding day shows higher elevations than 100° Fahrenheit.

In determining the size of the doses, when less than the full single dose for the age-period is to be given, it is our rule to administer first one-fourth of the full amount. If no general reaction follows, indicated by a temperature rise to above 99 2/5° F., we give the full dose five days later, whereas, if a general reaction is experienced to the first dose, with a temperature rise higher than 99 2/5° F., but not exceeding 101.5° F., we only double the first dose and then double once more. If

the temperature was higher than 101.5° F., we repeat the first dose and, according to the reactions observed, we give the full dose on the third and last administration. If the reaction to the second dose is again accompanied by marked fever, we repeat this dose and we give one or more additional doses in such cases, according to the intensity of the reactions. We administer all subsequent doses, after the first one, at intervals of five days, counting in cases of general reaction from the day on which the fever has subsided.

We deviate from this schedule in exceptional cases where the general reactions are prolonged and accompanied by temperature-rises which do not recede to normal in the course of forty-eight hours, and when there are decided focal reactions in the lung, persisting for forty-eight hours or longer. If, in cases of this kind, the full dose has not already been given, we repeat the last dose, administering in some instances a fourth or even a fifth dose, if like prolonged general or focal reactions recur.

We demand the return of the vaccinated children on the following day, at the same hour, for the purpose of inspecting the local effect at the site of injection, the temperature-chart, and accessible lymph-glands. When physical signs, however slight, were found on the first examination, we also examine the chest for focal reactions, and on dismissal we request the opportunity to examine the children again at a later period, setting the time according to convenience.

Children who have not reacted locally at all, after a full dose, we consider to be free from tuberculosis. Those who have reacted only locally we believe to have experienced an infection, but to be free from clinical disease; and all those who have developed general and focal reactions, or both, we look upon as tuberculous, and we keep them under observation for as long a time thereafter as possible.

THE DIAGNOSTIC VALUE OF THE VACCINE

In our earlier work we applied the tuberculin test as an aid in differentiating between normal, suspected and tuberculous children, using v. Pirquet's method in young children, and the conjunctival or the subcutaneous tuberculin tests in older chil-

dren and in adults. When it became evident that the vaccine itself could supply the information obtainable from these tests, and that it did so with greater certainty and uniformity than tuberculin, we gradually abandoned preliminary diagnostic tests entirely, thereby not only saving time, but simplifying our method as well.

It is generally conceded that a positive reaction to a test with any kind of tuberculin indicates that, in the tested and reacting individual, tubercle bacilli have found access to the tissues at some time or other before; but it is also admitted that a negative outcome of the test does not, in itself, prove the contrary. This observation applies particularly to the cutaneous tests of v. Pirquet and of Moro, as well as to the conjunctival test of Wolff-Eisner and of Calmette, none of which give information of additional clinical value, namely, as to the presence of actual tuberculosis or its extent or distribution; as to whether it is latent, active, healed, and so forth.

This information being at times very important, some observers have attempted to base more or less of an inference upon the degree of the observed reaction, taking into consideration the time of its appearance and subsidence, also the size, color, induration, etc., of the reacting area. The results recorded thus far have failed in all these attempts to afford sufficient light by which it could become possible to differentiate between active, latent or healed cases of tuberculosis.

In realization of this, it has been proposed to limit the v. Pirquet test to young children, and to base conclusions upon the assumption that, in them, tuberculosis is more often progressive and that the younger the child is, the less is the presence of healing or healed lesions to be expected. Hence, in very young children the occurrence of such healing or healed lesions was denied entirely, on the supposition that the natural resistance of small children is slight and, in any case, because the time is too short for healing to have occurred in them.

We do not share this view, believing as we do that healing, as well as progression of tuberculous processes, may occur at any age, since the conditions for the one or the other course are not so much governed by age as by the individual resistance, both general and specific, the latter of which is capable of being transmitted as well as of being acquired. In support of this

view we can draw upon our personal experiences, first in the demonstration of specific antibodies (including specific amboceptors for the tubercle bacillus) in the sera of very young children who did not react at all to the v. Pirquet or to any other tuberculin test; and then in the failure of general reactions to occur even to large subcutaneous doses of vaccine. In these children the local reactions at the sites of the injection of the vaccine presented the only evidence pointing to a previous actual contact with the virus of tuberculosis.

The conception of the all but absolute non-resistance of infants to tuberculosis-infection has long been held generally and has rarely been questioned until recent years. Franz Hamburger [2] who found in nurslings up to six months of age the tuberculosis-mortality to be one hundred per cent, and at the age of six to twelve months eighty per cent, claims that healed tuberculosis is found very rarely in children. He justly insists that little children should be kept absolutely from all contact with persons who have bacillary sputum, relating a case, by way of illustration, in which a child (age not given) was together in the same room with a consumptive for only half an hour and died a few weeks later of tuberculosis. While the author cites this instance as showing the great susceptibility of young children to infection with tubercle bacilli, and their lack of resistance, we are more inclined to view it as a case of superinfection of an already infected organism in which no immunity had as yet developed.

Other writers, for instance, Engel,[3] Bandelier & Röpke,[4] Koplik,[5] Czerni,[6] appear to share this opinion concerning the non-resistance of young children to tuberculosis-infection.

The observation of positive results of the v. Pirquet and of other tuberculin tests, in the absence of manifest symptoms of tuberculous disease, even in infants, make it evident, however, that an infection with tubercle bacilli need not be followed imme-

[2] Franz Hamburger; Wien. klin. Woch.; 1907, XX, 1069.—Med. Klinik; 1915, XI, 34.

[3] Engel; Beitr. z. Klin. d. Tuberkulose. 1909, XIII, 245.

[4] Bandelier & Röpke; Die Klinik der Tuberkulose. Würzburg, 1911, 84.

[5] H. Koplik; Johns Hopkins Hospital Bulletin; 1912, XXIII, 113.

[6] Ad. Czerni; Arch. f. Kinderheilk.; Bd. 60, 61; S. 242. cf. Intern. Centrlbl. f. d. ges. Tuberkulose-Forschung; 1913-14, VIII, 4.

diately by clinical disease. The frequent finding of pulmonary cavities in very young children indicates that a chronic course is possible in them, similar to that observed in older children and in adults. Degrees of resistance, equal to that of adults, have been demonstrated quite often by autopsy-findings in infants and young children who had died from other causes, in which latent or even healing or healed tuberculous foci were an accidental discovery.

According to these and to our own observations, a positive cutaneous reaction to tuberculin permits, therefore, no conclusion, even in very young children, other than that the tissues of the individual in question have, at some previous time, come in contact with or have been invaded by tubercle bacilli in sufficient numbers to produce sensitization; but whether or not this has resulted in actual disease, whether or not the latter has healed and become obsolete or is still lingering, or whether it is progressive, a positive cutaneous tuberculin reaction does not reveal, and in this respect we must base our clinical judgment upon other evidence.

The subcutaneous test with diluted old tuberculin, as a rule, causes no well-marked local reaction at the site of injection, and we depend upon the occurrence of general and of focal reactions for our positive diagnostic information; we rely especially upon the general reaction and more particularly upon a marked rise of the temperature. Inasmuch, however, as tuberculous tissue must probably be present for general and focal reactions to occur, such a positive reaction has the clinical significance that the lesion, however small it may be, is not a healed or obsolete one and that it may sooner or later progress and lead to clinical disease if this is not already present.

In our additional studies and observations of reactions as they occurred in our cases since we made our first report in 1912, we find that, in all cases which react positively to the v. Pirquet test, the vaccine causes local cutaneous reactions at the sites of injection, and that it induces also general and focal reactions, in like manner as does tuberculin. It can therefore take the place of both tests and it enables us, at least approximately, to differentiate between cases that may be considered as having acquired an infection which was apparently resisted and overcome by nature's own efforts, and others in which the

resistance was insufficient and nature's efforts had failed or had not yet accomplished the healing of the lesions which followed the infection.

In our interpretation of general and focal reactions which followed upon the subcutaneous injection of vaccine, as they occurred in relation to the size of the doses which produced them, we have considered the feasibility of deriving additional information as to the presence of more recently formed tubercles or of their probable absence; our observations indicate that children who react to small doses, as a rule are those whose general health, and especially whose nutrition, has been impaired more or less, and whose temperature is not persistently normal, whereas this is not the case, or at least does not coincide so often when the reactions are missed after small doses, and occur only after the injection of a full dose.

We may mention an illustrative case from our own clinical material concerning a child eighteen months old, of apparently healthy parentage, of normal appearance and weight. This child had never been seriously ill. Temperature, pulse, and respiration were normal; we found no enlarged lymph-glands; no physical signs in the chest; there was no history of a known exposure to infection. An examination of the blood showed agglutinins positive in a serum dilution of 1:20; complement-fixation test negative; v. Pirquet test positive. A full dose of vaccine, for the age period, gave a positive local reaction but was without effect otherwise, no rise of temperature being noted at any time during the forty-eight hours following its administration. Although specific agglutinins were present and the cutaneous reactions were unquestionably positive, indicating that tubercle bacilli had been taken up into the tissues at some prior time, and, in spite of the tender age of only eighteen months, we hold that this child had acquired no tuberculosis in a clinical sense and that, if tubercle bacilli had actually made their entrance, the infection had failed to develop for lack of sufficient virulence, or that it was checked and healed or had become obsolete, even at this early period of life, without causing any disturbance in health and without leaving any evidence which could be demonstrated by physical examination.

Our experience was different, however, in the cases of children who responded to small doses with a general reaction indi-

cated by fever, in addition to the local reaction at the site of injection of the vaccine. In the great majority of these children we were able to find evidences pointing to actual tuberculosis. As a rule there was a history of exposure in the family; often one of the parents had active tuberculous disease. We rarely failed to find enlarged lymph-glands; often we discovered physical signs as well as focal reactions and, in the majority of these children, the nutrition was not satisfactory; even those whose weight might be accepted as normal were delicate in appearance, often lacking in a good color of the skin, and sometimes appearing thin in proportion to their height and wanting in the rounded outlines of normal, healthy children. Such children are tuberculous, and we would hold to this opinion even though we had made no tuberculin test at all; always provided that we had looked for and eliminated other possible causes that might account for the abnormal findings. We would reconsider our opinion only in cases in which we failed to obtain a general reaction to a subcutaneous test with a full dose of tuberculin or vaccine, or after we had convinced ourselves that antituberculosis measures in the form of vaccination had not been followed by marked improvement. At the present time, however, and with the support of a considerable experience, we must state that such an exceptional case has not yet come to our notice. All our cases of this kind reacted with more or less rise of temperature, and they did so invariably when the first dose administered was a full one, or when the amount of the first very small dose was increased decidedly on a second administration.

If we are correct in our observations and conclusions, it follows that it is hardly worth the while to make cutaneous tuberculin tests, even in young children, inasmuch as negative results prove nothing and positive results give no information that is of clinical value. If then we were to make a test at all, we would prefer the subcutaneous method in which the question of non-absorption is, moreover, eliminated. In instances where such a test is needed to clear up or confirm a diagnosis, a negative result deserves consideration on its own part.

With a view of avoiding severe reactions, most physicians make the subcutaneous tuberculin test cautiously, giving first a mini-

mal dose and then increasing the amounts more or less rapidly until the maximum is reached. As a rule, three or four doses are administered in this manner. A general agreement as to the size of the maximal amount to be given as a single or as a final dose does not seem to exist. Koch recommended one milligram of the old tuberculin as a beginning dose, and his sequence of doses was 1—5—10—10 mgr. at intervals of one to three days. If a reaction occurred to any one dose, the next one, if it was given at all, was not to be increased, but the former amount was to be repeated. Persons who did not react to the repeated administration of ten milligrams of tuberculin were free from tuberculosis, in his opinion. Petruschky [7] diminishes the diagnostic doses materially and administers the sequence of 0.1—0.5—2—5 mgr. Pottenger [8] adheres to Koch's initial dose of 1 mgr. in the average adult, giving 3 or 5 mgr. after two days, if there is no reaction, and 7 or 10 mgr. three days after this. If the patient is a child, or an individual whose vitality is lowered, he usually begins with 0.1 mgr. and then increases to 1—3—5 mgr. While he realizes that a patient might react to ten, who does not react to five or seven milligrams, he feels that caution is warranted for the sake of safety. As to the possible danger from the initial dose of one milligram, Pottenger's experience of sixteen years, and also our own even longer experience, has proved that these amounts are not too large.

Personally we prefer to follow Koch when using old tuberculin in cases in which we anticipate no reaction; in delicate individuals and in children we proceed similarly to Petruschky, with the difference that, in adults, our final dose is always ten milligrams, because we have found that, if a reaction has not occurred to five milligrams, it is not apt to be a severe one if this dose is doubled.

Since we use the vaccine for diagnostic tests, instead of old tuberculin, we prefer to give a single, full dose in all cases in which the evidence elicited by our preceding physical examination suggests the probability that no tuberculosis is present or that any existing tuberculous foci are healed or obsolete, and that no severe general reaction will follow; we reserve the

[7] J. PETRUSCHKY; *loc. cit.*, S. 15 (p. 136).

[8] F. M. POTTENGER; Tuberculin in Diagnosis and Treatment. St. Louis, 1913, 26.

administration of repeated smaller and gradually increasing doses for other cases in which our previous findings lead us to look for the reverse.

When we first resorted to the prophylactic vaccination of children, we soon found instances in which the cutaneous tuberculin test was negative, whereas a positive local reaction developed at the injection-site of the vaccine, which was accompanied by fever in some cases; in instances of subcutaneous and conjunctival tests, on the other hand, the reactions to both preparations corresponded more closely, differing only in so far as the general reaction to old tuberculin, when this was given subcutaneously, appeared frequently to be more severe and as it persisted longer.

Since the great majority of instances, in which the tuberculin test is at all justifiable, are infections and latent lesions without marked clinical manifestations, they form a class in which we applied from one to three doses of vaccine, with results that were so satisfactory that they would be considered as most gratifying if they had followed after months, or even after years, of continuous hygienic, climatic, dietetic or other non-specific methods of treatment. This being the case, and the vaccination having served equally well for purposes of diagnosis and of treatment, it follows that the same results would be secured in similar cases, and in the hands of other observers, if the old and other kinds of tuberculin were to be replaced by a whole antigen, in solution and properly balanced, like our vaccine. While we make the specific diagnostic test, we treat these early infections and latent lesions successfully at the same time, and thereby protect the organism against the development of active disease; or, putting it the other way, in the therapeutic or prophylactic application of the vaccine, in this class of cases, we are at the same time put in possession of all diagnostic information that any tuberculin test can supply.

SPECIFIC REACTIONS

Our children's material was derived almost exclusively from tuberculous families; in many instances exposure had been prolonged, and often it was still continuing daily. Since publishing

our first report, we have completed the vaccination of 1512 additional children and of 106 adults, and we have complete records of both local and general reactions, the latter with temperature observations before and after the administration of the vaccine, in 1315 individuals of all ages from four weeks to sixty years.

In this entire material we find that only twenty-five individuals, or 1.7 per cent, failed to react locally to a full dose of the vaccine, namely, twenty children and five adults, and no general reactions were observed in them after the administration of this dose. In the remainder of 1290 vaccinations, the reactions were only local in 366, or 28.3 per cent; and they were attended by more or less fever and in some instances by focal reactions, in 924, or 71.6 per cent, of the cases.

We vaccinated 726 individuals with a single, full dose, observing reactions as follows:

No general reaction followed in	278	or 38.2	per cent
There was a general reaction with			
fever not exceeding 100° F. in	205	" 28.2	" "
" " " 101.5° F. in	158	" 21.8	" "
" " " 103° F. in	63	" 8.7	" "
" " " 104° F. in	22	" 3.0	" "

In the remainder of the vaccinated persons, two, three or four doses were administered, accordingly as the first (trial-) dose had produced little or no reaction.

We made 392 vaccinations with two doses, the first one being one-fourth, the second being the maximal dose for the age period.

No general reaction followed in	78	or 19.9	per cent
General reaction after 2nd (full) dose only	122	" 31.1	" "
" " " 1st small dose only	102	" 26.0	" "
" " " 1st and 2nd doses	90	" 22.9	" "

In the 90 cases in which fever followed after both doses, it was higher after the first dose in 50; it was of the same degree both times in 12; and it was higher after the second dose in 28 of the cases.

We made 134 vaccinations with three doses. The first dose was usually one-fourth, the second dose one-half, and the third dose the full amount for the age period.

No general reaction followed in	10 or	7.46	per cent
General reaction after 1st dose only	44 "	32.83	" "
" " " 2nd dose only	19 "	14.18	" "
" " " 3rd dose only	4 "	2.98	" "
" " " 1st and 2nd doses only	34 "	25.30	" "
" " " 1st and 3rd doses only	13 "	9.70	" "
" " " 2nd and 3rd doses only	00 "	0.00	" "
" " " all 3 doses	10 "	7.46	" "

In cases where two or three general (fever-) reactions occurred,

The fever was highest after the 1st dose in	12
" " " " " " 2nd " "	29
" " " " " " 3rd " "	4
" " " the same after the 1st and 2nd doses in	2

We made 38 vaccinations with four doses; the doses being approximately like those in the preceding group, except that the third dose was increased to three-fourths of the full dose, or one of the doses was repeated.

No general reaction followed in	0 or	0.0	per cent
General reaction after the 1st dose only in	3 "	7.89	" "
" " " " 2nd dose only in	5 "	13.16	" "
" " " " 3rd dose only in	14 "	35.84	" "
" " " " 4th dose only in	1 "	2.63	" "
" " " " 1st and 2nd doses in	4 "	10.52	" "
" " " " 1st and 3rd doses in	9 "	23.69	" "
" " " " 2nd and 3rd doses in	2 "	5.26	" "

The fever was highest after the 1st dose in	6
" " " " " " 2nd dose in	9
" " " " " " 4th dose in	1

In the comparative study of the degrees of fever accompanying a general reaction, accordingly as we gave a single dose or several increasing doses, we found that, contrary to our expectations, not enough difference was to be noted to justify any conclusions.

We were occasionally surprised in both series by results which were entirely opposite to those which we had expected. Nevertheless, we appreciate that the reactions might have been more severe in the groups receiving two or more doses, if only the full single doses had been administered.

INFLUENCE OF REACTIONS

Of great interest appear to us our observations of the influence of marked focal and general reactions upon the clinical results. After the first introduction of tuberculin, most of those clinicians who continued its use took pains to avoid marked fever-reactions, and we also extended this precaution to other preparations and practised it in the use of our own products.

Our clinical material has always been of such a nature that occasions to make a diagnostic tuberculin test were infrequent. When in doubtful cases its advantages were desired, we usually followed the gradual method of Koch, mentioned above. There were but few children among our patients and, in the majority of them, the disease had advanced beyond the early stage, so that it was not until we undertook our studies in prophylactic vaccination that an opportunity for a systematic study of reactions was afforded us, which yielded the results described above.

While we cannot say how these results might have differed had old tuberculin been given subcutaneously instead of the vaccine, we certainly have seen no harm following the reactions; on the contrary, the conviction became unavoidable that, in cases in which a general reaction occurred which was accompanied by a focal reaction, or when marked focal reactions occurred without any, or with only a slight rise of temperature, more rapid improvement, both local and general, was usually observed than was the case when no focal or general reactions occurred at all.

It may be of interest to the reader to relate a case in point, it being one of the group vaccinated with four doses, in which no reaction, either focal or general, had followed after the administration of the first three doses. The case was that of a young woman whose examination showed marked physical signs involving the right upper lobe and the apex of the left lung. She was practically free from fever, but her general health was indiffer-

ent, her weight was decidedly below normal and had always been so. There was tuberculosis in her family, one or two deaths having occurred, and a brother was a patient in the Winyah Sanatorium. The extensive lung involvement suggested the exercise of caution, hence the first dose given was only one milligram which caused a local reaction. The second and third doses were two and four milligrams, respectively, with the same result. At that time the patient desired to return to her home; an examination indicated a moderate degree of improvement in physical signs; she had gained a few pounds in weight, and her serum tests showed an ample degree of antibodies.

On account of the rather extensive lung involvement, it was decided that she should be given one more dose, and this was increased to eight milligrams. To this dose she reacted promptly, both locally and generally; her temperature reached over 103° F. in the course of eight hours, but returned to nearly normal by next morning and had become entirely so that afternoon. An examination of the lungs, in the forenoon, showed a focal reaction which was unmistakable; it extended considerably beyond the limits which had, before, been believed to be involved and was attended by a slight cough, especially noticeable on deep inspiration. At her reexamination, ten days later, it was impossible to detect anything abnormal in the chest. The patient herself insisted upon a state of well-being more satisfactory than she could remember having experienced before, and she particularly mentioned a sense of relief from oppression, and of feeling so free in her chest that "it was a pleasure to breathe."

When cases with a similar degree of lung involvement had been found among children and adults in 1911, we had, at first, no expectation of exerting any decided curative influence with one or two doses of vaccine, and assumed that prolonged treatment would be required. Among the vaccinated children at the Thomasville Orphanage there were a number of these cases and, although they each received two doses, it was not anticipated that the lung-lesions would undergo retrogressive changes to the degree which Dr. Julian found and reported to us about six months later. His observations were so remarkable that one of us personally went to Thomasville and examined the children with Dr. Julian, finding indeed that, in some of them, the previously recorded physical signs had entirely disappeared, while

they had greatly diminished in others. The same observation was made in respect to enlarged lymph-glands, and there was likewise a remarkable general improvement, with a gain in weight in some of the children, which greatly exceeded the normal rate of increase. We then reexamined a number of children, vaccinated by us in the summer of 1911, with similar results. We have already (p. 138) referred to Dr. Julian and to other physicians who have used the vaccine in this class of cases, the results in which, together with our own continued experience, leave us no doubt that a few doses, or even a single dose of the vaccine, given at a comparatively early period of tuberculosis, cause the retrogression and healing of tuberculous lesions, and that, as far as we are able to judge, the rapidity and degree of the improvement often appear to stand in relation to the FOCAL REACTION on the part of the tuberculous tissue, which is frequently attended by a general reaction and by fever.

Although the avoidance of marked general reactions is no longer such a matter of solicitude on our part as it was several years ago, when we did all we could to prevent them in the treatment of even early-stage cases, we cannot overcome our fear entirely that sooner or later we may meet with a case in which we might have occasion for regret, were we to produce marked reactions deliberately by giving larger single doses than necessary, even in cases where the evidences indicate only latent or comparatively quiescent tuberculosis of internal organs. In calling attention to our observations made after such reactions, which came to our notice unexpectedly, we hope that, with the aid of others who meet with similar cases, we may in time determine whether or not the fear of severe general reactions is at all justified.

If we are right, as at present we believe to be, at least in so far as we are of the opinion that marked focal and febrile general reactions are not harmful, our method of vaccination will become a still simpler procedure, because in that case the single full dose will be the proper one to administer to any child or adult whose vaccination it would be at all justifiable to consider. On general principles, febrile general reactions, even with temperature-rises to 103° F. and over, cannot be said to be objectionable; at least, they are countenanced in the case

of prophylactic vaccination against typhoid fever, in which these reactions are sometimes much more severe than we have observed them, as a rule, in the use of our vaccine. In vaccination against smallpox also, very severe fever-reactions are noted at times, and more or less marked fever-reactions occur in the use of other bacterial vaccines and are, in fact, expected if not desired.

Inasmuch as our present experience, as here given, differs more or less from that of some other observers, we desire to mention the views of a sufficient number of experienced clinicians, to enable the reader to take them into consideration, calling however his attention to the fact that the observations were made during the employment of various tuberculin preparations and tubercle bacillus emulsions, and not with our vaccine. We wish to state further that the opinions to be cited are usually based upon the effects of therapeutic doses, administered to patients who may be presumed to have represented a more marked stage of the disease than we included in those of our cases which we selected for prophylactic vaccination, especially when we administered a single full dose of the vaccine. It is of course quite possible to avoid more severe degrees of fever and well-marked focal reactions with the vaccine, if the doses are small enough and if they are increased slowly, as is our practise in patients whose disease has advanced further. At the time when we shared the fears which some clinical observers still entertain, it was our custom to begin the treatment of manifestly tuberculous patients with doses representing about one-hundredth to one-tenth of those which we now employ, and to repeat each dose at least once, even if no reaction was observed, before increasing it. Although we never could prevent reactions entirely, those which we did observe with that mode of procedure were, as a rule, slight. We further call attention to the fact that in some of the individuals, whose reactions were general and were accompanied by fever, physical signs were not yet present or, if they were in evidence, they were comparatively slight; also that all reactions occurred in cases in which a diagnostic test would have been proper and necessary; and none were among them in whom the results of examination would have justified the inference that open lesions were present, or that a tendency existed in the lesions to break down.

In regard to the fear of various clinicians, of general reactions in the therapeutic use of tuberculin, it may be said that, in order to be consistent, those who consider these reactions as seriously dangerous must take the same attitude toward the diagnostic employment of tuberculin, in which the doses are increased purposely and rapidly in order to produce reactions, the degree and intensity of which can never be foretold with certainty.

One of the early objections to tuberculin was raised on account of the focal reactions in the form of pneumonias, which were found on autopsy and which served Virchow in his opposition to the remedy during his long-continued demonstrations before the Berlin Medical Society in the early months of 1891. The fear of these so-called "injection-pneumonias" became practically universal and everywhere caused the abandonment of tuberculin as a therapeutic agent. The medical profession appeared at the time to have lost all independent judgment, forgetting that pneumonic changes are common findings in progressive cases of phthisis and that they constitute a frequent immediate cause of death. Since the resumption of tuberculin therapy, very little, if anything, has been heard of "injection-pneumonias," although severe reactions have not been eliminated entirely by a more conservative dosage.[9]

The fear of reactions was intensified by the publication of

[9] The writers have often wondered whether the change during the winter of 1891, in the manufacture of tuberculin, then called "Koch's Lymph," was not a factor in the causation of reaction. It is not generally known that "Koch's Lymph" was produced more directly from tubercle bacilli than was the case after a change in its production had become necessary by the enormous demand for it which could not be met at first. As first prepared, the tubercle bacilli were grown on solid media from which they were removed, and then extracted in 5 per cent glycerin-water suspension with heat, until the fluid was concentrated to one-tenth of its bulk; after filtering, it was ready for use. In order to obtain tuberculin in greater quantities, the tubercle bacilli were then grown upon glycerin-bouillon containing 5 per cent glycerin; the matured culture was subjected to concentration by the same degree of heat, and, after filtering, the product was and is called "Koch's Tuberculin." In our opinion the two preparations were not necessarily identical in the content and in the nature of specific bacilliary substance; and tuberculin contains, in addition, the non-specific proteins of the culture medium, which were not present in the "lymph."

Liebmann,[10] who reported that he found tubercle bacilli in the blood of eight persons treated with Koch's remedy. Guttmann & Ehrlich [11] thereupon examined twenty-nine cases under treatment with tuberculin, with entirely negative results. In the following years the question of the "mobilization" of tubercle bacilli by tuberculin was brought up frequently, and Lydia Rabinowitsch [12] showed definitely that tubercle bacilli may be found in the blood, in the course of tuberculin treatment. The direct relation of this occurrence to tuberculin had to be reconsidered, however, when like observations were made with increasing frequency, by different observers, even in the blood of afebrile and non-progressive cases that had not been treated with tuberculin or with other specific bacillary products.

In our observations based upon numerous blood-examinations for tubercle bacilli, in all stages of pulmonary tuberculosis, by the methods of Jousset and of Rosenberger, we found them with great frequency in instances of softening and progressive excavation: that is, in cases in which we consider specific treatment to be contra-indicated. Their advent in large numbers, as a rule, is signalized by an abrupt rise of temperature which often presents a remittent type during their presence in the circulating blood. We have made this observation so often, after the occurrence of such abrupt temperature-rises or of changes in the type of the fever in the more advanced stages of phthisis, that we not only look for a pneumonic complication, but we also examine the blood for tubercle bacilli. The time of their continuance in the circulation appears to vary and we have found them repeatedly and daily over periods of weeks or longer in cases of rapid softening. On the temporary or permanent disappearance of tubercle bacilli from the blood, the type of fever as a rule changed to an intermittent one with lower maxima. We have made such observations also in instances of the first occurrence of caseous softening, in patients who had as yet no open lesions and therefore no bacillary sputum, and found that the bacilli disappeared soon after the focus had secured an exit into a bronchus, with the amelioration that usually follows in the febrile and other symptoms.

[10] V. LIEBMANN; Berl. klin. Woch. 1891, XXVIII, 97.
[11] P. GUTTMANN & P. EHRLICH; Deut. med. Woch. 1891, XVII, 251.
[12] LYDIA RABINOWITSCH; Tuberculosis. 1913, XII 479; and elsewhere.

In our judgment the accompanying symptoms, including fever and its increase or its change in type, represent intensified specific reactions, depending upon and corresponding in degree with the amount of soluble and corpuscular antigen which enters the blood, and we have been able to stop this spontaneous intake of antigen by lung-compression, which was followed by a prompt relief of symptoms and by the disappearance of the bacillemia.

It will be remembered that, in his first communication on tuberculin, Koch [13] expressed the opinion that the action of the remedy is exerted not upon the tubercle bacilli, but upon the tuberculous tissue; that it promotes the softening (*"Einschmelzung"*) of the latter and, by the removal of the pathological tissue, places the organism in a condition in which healing can occur. Reactions were therefore believed to be useful and indicative of the direct action of the remedy upon the disease-process. Many observations of real or supposed harm resulting from pronounced and repeated reactions suggested that Koch had erred, if not in his conception then in its application, and as early as 1891 recourse was had to small and even minute doses by which fever-reactions, and even manifest focal reactions, were to be avoided. With the reestablishment of tuberculin-therapy the gentle, "insinuating" method, according to Sahli, was practised almost generally. But even at that early period the relation between reaction and improvement was noted. So said Koch,[14] "after every tuberculin-reaction an unmistakable improvement follows in the tuberculous process." Professor v. Leyden [15] believed that the fever-reaction constitutes an essential part of the intended curative action of tuberculin; Königshöfer & Maschke [16] say, in reporting both general and focal reactions in all cases of tuberculous eye disease treated with tuberculin, that "in consequence of this reaction" considerable improvement was observed in all cases, and complete cure in corneal ulcers.

Further studies in infection and in immunology showed that a definite relation exists between the reactibility to tuberculin

[13] R. Koch; *loc. cit.* (p. 117).
[14] R. Koch; Deut. med. Woch. 1897, XXIII, 209.
[15] v. Leyden; Klin. Jahrbuch. Ergänzungsband. 1891, S. 5.
[16] O Königshöfer & E. Maschke; Deut. med. Woch. 1891, XVII, 72.

and the development of immunity in tuberculosis. In several instructive communications, v. Pirquet & Schick [17] illustrated this relation and arrived at the conclusion, which was confirmed by Hamburger,[18] that the reaction to tuberculin depends upon the presence of specific antibodies. An individual that possesses no antibodies does not react to tuberculin. This may mean either that this individual is entirely free from tuberculosis or that all available antibodies are bound to the bacterial substances, as is the case in generalized or far-advanced tuberculosis and in the acute disease.

This idea was developed further, especially by Wolff-Eisner,[19] Römer,[20] Hamburger,[21] who showed the intimate relation between the condition of hypersensitiveness, following upon a first infection or sensitization, and the reaction to the specific bacterial proteins (tuberculin), as well as to immunity. It was asserted that hypersensitiveness is a necessary condition for the occurrence of a reaction and also for the development of immunity, and that, as Hamburger expresses it tersely, a positive reaction to tuberculin is an indicator of tuberculosis-immunity. Wolff-Eisner concluded that a brisk, sharp reaction, which subsides as promptly as it develops, indicates a satisfactory degree of immunity, or one which can be increased by treatment and therefore is prognostically favorable; while the absence of a reaction in manifest tuberculosis, or its slow, protracted appearance and course denote the reverse and are prognostically unfavorable. In other words, a certain degree of hypersensibility, a power of reaction or a reactibility is a condition for the development of immunity.

These views, which were supported by experimental evidence and by clinical observation, make the problem of tuberculin-reactions appear in a new light and once more prove the deep insight and the comprehensive, if more prophetic, understanding of Koch, of the new science of immunology of which he had

[17] v. Pirquet & B. Schick; Wien. klin. Woch. 1903, XVI, 758 and 1244; 1905, XVIII, 431.

[18] Hamburger; *ibidem*, 1908, XXI, 1043.

[19] Wolff-Eisner; Beitr. z. Klin. d. Tuberkulose; 1908, IX, 1. Also his monograph, Würzburg, 1909.

[20] P. H. Römer; Beitr. z. Klin. d. Tuberkulose. 1908, XI, 79 (99).

[21] Franz Hamburger; *loc. cit.*

been one of the founders. Building upon these views, a broader and more teleological conception of the tuberculin reaction in its different phases has been developed.

It is to be noted that the opinions concerning and the explanations of the action of tuberculin and of its very nature are by no means unanimous. Wolff-Eisner [22] declares that all tuberculins, from old tuberculin to bacillus emulsion, are essentially alike and that they act through the presence of corpuscular substances which give rise to the formation of bacteriolysins in the blood. Van Calcar [23] denies this, and we ourselves [24] are inclined to persist in the opinion which we expressed several years ago, according to which the specific products of tubercle bacilli, which are available on the market, fall into two groups: namely, the tuberculins proper, i.e., those which are made from the culture-media upon which the bacilli have been grown and from which they have been removed by filtration, and those which contain the bacillary body-substances. Our experimental investigations, which will be described in Part III, have convinced us that the tuberculins proper, which include Old Tuberculin, Albumose-free Tuberculin, Bouillon Filtré, Endotin, Béraneck's Tuberculin, and many others, represent *exotoxins* of tubercle bacilli, that is to say, products of secretion or of metabolism of the bacilli, which enter the culture-medium during their growth upon it. If the cultures are permitted to become old before filtration, or if the tuberculins are prepared with the aid of heat, they contain, in addition, certain amounts of the bacillary extractives. The second group embraces the preparations in which the bacilli themselves are contained, viz., Koch's Tuberculin R. and his New Tuberculin or BE, and the bacillus emulsions of others; also extracts of the bacilli, like our Watery Extract, our Vaccine, Landmann's Tuberculol and some others. These preparations differ from the tuberculins proper in that they contain bacillary *endotoxins*, i.e., the body constituents of the bacterial cells.[25] The custom of designating

[22] A. Wolff-Eisner; *loc. cit.* S. 228, ff. (p. 101).

[23] R. P. van Calcar; Tuberkulose und Immunität. Leiden, 1910, S. 105.

[24] K. v. Ruck & S. v. Ruck; *loc. cit.* (p. 87).

[25] While all these preparations are capable of producing reactions, as is claimed by Wolff-Eisner, they do not do so interchangeably in the sense

all preparations of this class, without distinction, as "tuberculins," must unfortunately often give rise to misunderstandings. In agreement with Citron [26] and others, we reserve the term for Koch's old tuberculin alone, which we believe to be essentially different from the preparations containing the tubercle bacillus substances. We are also in accord in this view with Hans Aronsohn,[27] who showed recently that a typical hypersensibility can be produced with tubercle bacilli and with solutions containing native tubercle bacillus proteins, but not with old tuberculin.

As to the focal action of tuberculin, many authors (Oscar Bail, Wassermann & Bruck, etc.) agree with Koch that the remedy acts directly on the tuberculous tissue. In Bail's [28] opinion, it causes the formation of a toxic reaction-product which stimulates inflammation around the tuberculous focus, while the general effect is not direct but indirect. Much & Leschke [29] approach Wolff-Eisner's theory when they relate that in all animals treated with tubercle bacilli, opened up by means of

that all necessarily produce reactions in the same tuberculous individual. The occurrence of the reactions undoubtedly depends upon the presence of specific amboceptors, but the several toxic substances differ in their chemical and molecular constitution, and the specific action of the amboceptors which are formed in response to their absorption is limited to those toxic substances which have given rise to their development and is not general for all tubercle bacillus products. A patient may, therefore, react to a secretion- or metabolic product of the tubercle bacillus, e.g., to Old Tuberculin, and fail to react to one or more of its body-constituents.

It appears that Wolff-Eisner's statement that the action of all tuberculins was identical, has led some physicians to accept his corollary view that, in consequence, it makes no difference in the development of immunity whether one or the other product of tubercle bacilli is administered. Such a view is undoubtedly erroneous and is certain to lead to disappointing results in the specific treatment of tuberculous patients. While it is true that all so-called tuberculins have the common action of producing specific amboceptors, it must be borne in mind that the amboceptors for one of the several specific products of the tubercle bacillus are not specific for all, and that specific amboceptors are required for the body-constituents and not for the exotoxins which the tubercle bacilli secrete in their living state, if a bacteriolytic immunity against the tubercle bacillus is to be produced.

[26] JULIUS CITRON; *loc. cit.* S. 50 (p. 92).

[27] HANS ARONSOHN; Deut. med. Woch. 1914, XL, 487.

[28] O. BAIL; Ztschr. f. Immunitätsforsch. 1912, XII, 451.

[29] H. MUCH & E. LESCHKE; *loc. cit.* (p. 48 footnote).

weak organic acids, an immunity was produced which was mainly bacteriolytic and which likewise found expression in a decided hypersensibility to intracutaneous applications of tuberculin. In Sahli's[30] opinion, tuberculin produces "toxi-immunity" (*Giftfestigung*), which renders the organism insensible to the proteid poisons elaborated in the tuberculous focus. Against this, Hans Aronsohn[31] claims that the action of tuberculin is neither immunizing nor does it produce a toxi-immunity, and that any favorable effect is probably due to a stimulating action which tuberculin in small doses exerts on the leucocytes. Accordingly, Aronsohn denies the specific character of the focal and general tuberculin-reactions (which had before him been disputed by Hüppe, Scholl and others), while admitting it for the skin-reaction. Tuberculin contains, in his opinion, a specific and a non-specific component, the same as Petruschky postulates for the hypersensibility of the tuberculous organism.

The hypersensibility of the tuberculous organism, to tuberculin, or to specific products of tubercle bacilli, which is a necessary condition for the occurrence of a reaction, is induced by the disintegration, in the organism, and by the absorption of the foreign, bacterial protein, to which the organism responds by the formation of reaction-products tending to bind the foreign protein, to reduce it to a simpler molecular state, and to neutralize its action. The reaction which follows the injection of tuberculin consists, according to Petruschky,[32] of four characteristic phenomena:

1. Local circumscribed inflammation,
2. Rise of body-temperature.
3. Active hyperemia in the tuberculous focus with increased focal temperature,
4. General symptoms of malaise, etc.

The beneficial action of bacterial vaccines depends, according to Wright,[33] upon the power of the tissue-fluids to break them

[30] HERMANN SAHLI; Tuberculin Treatment (English by W. B. Christopherson, from the third German edition). New York, 1912.—Ueber Tuberkulinbehandlung. Basel, 1913. Vierte Auflage.

[31] HANS ARONSOHN; *loc. cit.* (p. 169).

[32] J. PETRUSCHKY; *loc. cit.* Grundriss, S. 15 (p. 136).

[33] A. E. WRIGHT and others; *loc. cit.* (p. 118).

down and to assimilate them; or, in other words, "before a vaccine can affect the tissues in such a way as to cause any immunizing response, the bacterial protoplasm must be brought into solution and probably also broken down. The power of the individual's body fluids to break down the vaccine (bacterioclastic power) explains the fact that reactions, both general and local, may follow the injection of vaccines of easily broken-down organisms, such as the typhoid bacillus; and also that, following the injection of a large dose of vaccine (e.g., tuberculin) into an infected patient (with an enhanced bacterioclastic power), a general reaction occurs."

The local reaction at the point of injection is generally considered to be of diagnostic value and is caused by the presence of free antibodies in the tissues, by which the injected antigen is anchored, leading to a circumscribed tissue-stimulation with hyperemia. As far as the therapeutic application of tuberculin preparations is concerned, J. Ritter [34] holds that the local reaction is of no import whatever. He denies the correctness of Spengler's assertion that it "announces" a focal or a general reaction, and claims that it occurs independently of both. It usually appears from six to twenty-four hours after injection and disappears gradually in the course of from two to four days. Treatment with old tuberculin tends to suppress the local hypersensibility to it, which, according to Bessau,[35] is not desirable.

The General Reaction is, as a rule, accompanied by fever, with or without malaise, aching in the joints, headache, nausea, etc.; but some of these symptoms may be absent or may occur without a marked rise in temperature or without any rise. The fever usually commences between eight and sixteen hours after the injection of tuberculin; it may begin sooner, or, according to Petruschky,[36] it may be delayed as much as two or three days. The return to normal takes place gradually, between twenty-four and forty-eight hours later.

We have never observed so long a delay as three days, and not even one of forty-eight hours, in which we could not, on careful investigation, eliminate the relation of the fever to

[34] J. Ritter; Tuberkulose-Fortbildungskurse. Würzburg, 1914, II, 49.
[35] G. Bessau; Münch. med. Woch. 1915, LXII, 323.
[36] J. Petruschky; *loc. cit.* S. 16 (p. 136).

the administered dose of the specific remedy or in which such a relation was not decidedly doubtful. Petruschky states that fever, which appears earlier than six hours after the injection, is always due to other causes than tuberculosis, and the same is claimed by him for continued fever, or for temperature rises and remissions with large variations continued through several days.

It is the fever-reaction to tuberculin and allied products to which most of the objections have been raised, and most phthisiotherapists advocate avoidance of doses or of their increase by which rises of temperature of over one degree Fahrenheit are caused. Others are, however, of different opinion. C. Spengler [37] considers fever-reactions to be a welcome index of strong focal reactions, and believes that healing is promoted by such reactions. Engel [38] states that the height of the reaction-fever is of less serious import than is its duration, and that it is the continued fever that tends to work harm. According to Rolly,[39] a moderate increase of temperature has a curative, rather than a harmful, effect, because it stimulates an energetic activity of the leucocytes and a greater production of immune substances. Ed. Aronsohn [40] points out that fever, unless excessive, does not injure the body-cells, but rather increases the formation of protective substances, and that fever probably increases the power of the antigens to bind, or to be bound to, the cell-receptors. On the other hand, Petruschky [41] claims that every-fever-temperature is "a cry of distress of the body" and indicates an excessive strain upon it. He asserts that the fever-reaction is not required in tuberculin treatment to secure therapeutic results.

Pottenger [42] calls attention to the fact that it is not the rise of temperature which is harmful, but the underlying condition which produces it, which at the same time causes the phenomena that often accompany and are carelessly attributed to it. According to him, a temperature reaction is a manifesta-

[37] C. Spengler; Deut. med. Woch. 1906, XXXII, 311.
[38] Engel; Beitr. z. Klin. d. Tuberkulose. 1909, XIII, 245.
[39] Rolly; Deut. med. Woch. 1911, XXXVII, 2121.
[40] Ed. Aronsohn; Deut. med. Woch. 1912, XXXVIII, 73.
[41] J. Petruschky; *loc. cit.* S. 67 (p. 136).
[42] F. M. Pottenger; *loc. cit.*, p. 111 (p. 156).

tion, on the part of the organism, of its ability to cope with some pathological process; the danger time, in an infectious disease, is not when the fever is high, but when it is low, because the failure to respond shows that the body-cells are exhausted and can no longer react to the stimulus of the toxins.

While Koch [43] admits that, in the treatment with specific remedies, fever-reactions should be avoided as much as possible, Sahli [44] concedes, in spite of his fear of them, that doses of tuberculin, which are large enough to cause such reactions, may occasionally have a better effect than those to which no fever-reactions have followed. Still, he insists that reactions are dangerous and should be avoided in practise. According to Schürer,[45] in rabbits that were first sensitized with human tubercle bacilli, and then treated with albumose-free tuberculin, antibodies were formed only in those animals which had reacted with marked fever. This he states is in accordance with Ehrlich's view, that fever-reactions are necessary for the production of antibodies.

The idea that fever is not necessarily harmful in itself, and that it may even be salutary, dates back to the time of Hippocrates and his pupils; it recurs in the writings of Sydenham, Stahl, Boerhaave, Friedrich Hoffmann, Schönlein, Canstatt, and is admitted, under exceptional conditions, by Virchow.

Whatever may be the net result of these opinions, which are representative of the many expressed in literature, it must be acknowledged that fever, as such, is no longer considered as something necessarily injurious which must be avoided and suppressed. It has been shown that fever constitutes a defense of the organism, a tissue-reaction to an exogenic stimulation or irritation; therefore, the very fact that the sick body can respond with fever to the presence of a noxious agent is an indication of battle shown against the enemy. We agree with the view that fever is more apt to work harm if it is long continued, and that even a very high fever need not be injurious, but that it may

[43] ROBERT KOCH, in BANDELIER-RÖPKE; Spezifische Diagnostik u. Therapie der Tuberkulose. 3te Aufl. Würzburg, 1909. Vorwort.

[44] H. SAHLI; Beitr. z. Klin. d. Tuberkulose. 1908, X, 366 (S. 370. Footnote).

[45] JOH. SCHÜRER; Deut. Arch. f. klin. Med. 1913, CIX, 112.

be rather the reverse, provided the temperature returns to normal within a reasonable time. This is suggested very strongly by our own observations of decided improvement in patients who responded with sharp rises of temperature to vaccination or to treatment.

Focal Reactions.—In the consideration of focal reactions, by other observers, it is well to remember that, according to the early conceptions of the action of tuberculin, curative results were apparently dependent upon these reactions. So reported v. Bergmann,[46] that in his cases of lupus, healing was more rapid after decided focal reactions; Cornet[47] declared a few years ago that, while the prevailing method of tuberculin treatment seeks to avoid severe reactions, it does not produce such surprising improvements as he used to observe in isolated cases with the large doses administered at first. In 1898, Carl Spengler[48] expressed the view that focal reactions are absolutely necessary to produce healing, if not accompanied by high fever. Citron[49] also found focal reactions to be necessary for healing, and Hamman & Wolman[50] consider it fair to state that these reactions promote fibrosis. Bandelier & Röpke[51] agree with others upon the importance of focal hyperemia, but insist that it may occur without malaise and fever. Pottenger fears repeated focal reactions as potentially harmful. In case of a small circumscribed lesion, he considers that a marked focal reaction, even if it is repeated, is not harmful; but, if the tuberculous process is decidedly active and more extensive, a marked focal reaction might be injurious, although as a rule it does not prove so if it is not continuous. He often noted marked improvement after decided focal reactions. Pottenger does not entirely agree with Wright's idea that repeated focal reactions are harmful because the organism is kept in a negative phase, thus preventing an immunizing response; he believes rather that, by repeated severe focal stimulation, the destruction of tissue

[46] v. Bergmann; Klin. Jahrbuch, Ergänzungsband. 1891, S. 238.

[47] G. Cornet; Die Tuberkulose. 2te Aufl. Wien., 1907, S. 1010.

[48] Carl Spengler; Beitr. z. Klin. d. Tuberkulose. 1914, XXXI, 127.

[49] Julius Citron; Berl. klin. Woch. 1907, XLIV, 1135.

[50] Hamman & Wolman; Tuberculin in Diagnosis and Treatment. 1912, New York, p. 255.

[51] Bandelier & Röpke; Lehrbuch der Spezifischen Diagnostik und Therapie der Tuberkulose. Würzburg. 7te Aufl. 1913, S. 160.

is forced, instead of a healing process being promoted. Bessau [52] believes that severe focal reactions are not so much injurious in themselves, but are so indirectly, the injury depending upon the sequelæ of the reaction, viz., general reactions or toxi-antianaphylaxis. In his personal experience, White [53] is quite sure that tuberculin has done him great good when he took enough at one time to produce a mild reaction, but that he has never been able to notice the slightest influence from small doses. Other English observers, for instance, Sir James Fowler,[54] unequivocally consider focal reactions dangerous.

Petruschky [55] summarizes the manifestations which are produced by focal reactions as follows:

1. A local hyperemia is induced in all visible tuberculous foci, after each introduction of tuberculin into the body.

2. An increase of the polynuclear leucocytes, and an increased activity of the phagocytes occur after even quite small doses.

3. The ability of the organism increases to break down tuberculin, i.e., to render it ineffective in itself; this is observed in continued introductions of tuberculin. It suggests biochemical processes in the blood, the formation of "antibodies."

4. In the favorable course of tuberculin treatment there is a diminution of the hypersensitiveness of the nervous system and an increase of trophic and regenerative processes in the body, which is manifested by an increased appetite and an increase in the body-weight.

The opinions to which we have just referred were formulated for the purpose of accounting for certain phenomena which seemingly stood in relation to the administration of tuberculin and of similar preparations in all stages of tuberculous disease, and especially in pulmonary phthisis. Aside from its bacterial origin, however, the course of phthisis is dominated by many factors, over which the specific treatment has no control whatever; its symptoms vary greatly under any method of treatment, and often change abruptly without any apparent external cause. We have no doubt that the clinical observations, which under-

[52] G. Bessau; *loc. cit.* (p. 171).
[53] W. Ch. White; The Lancet; 1913, II, 377.
[54] Sir James K. Fowler; The Lancet; 1913, II, 376.
[55] J. Petruschky; *loc. cit.* Grundriss, S. 46 (p. 136).

lie these and other opinions, have often been misinterpreted, and that specific treatment has often been either credited or charged with manifestations and results for which other conditions were really responsible, a viewpoint which is supported by the opposing opinions of different observers.

Every physician who has had much experience in the specific treatment of pulmonary phthisis has had occasion to modify and remodify his views concerning the action of the particular preparation which he employed, its dangers and its clinical benefits. In order to secure a basis for valuable and valid conclusions in this respect, it is first necessary to study uncomplicated and purely tuberculous processes, as they are met with in the earliest manifestation of the disease. We believe that our observations upon our vaccinated children and adults are, therefore, peculiarly adapted for the study of the question under consideration, and that the early-stage closed cases of our clinical material are likewise suitable for this purpose. Since we have seen no harmful, but as a rule only beneficial, results following the occurrence of general and focal reactions in a comparatively large number of cases, we believe ourselves justified in the assumption that in this class of cases the contrary must be comparatively rare. We shall have occasion to consider this subject further in connection with the specific treatment of tuberculosis, and on p. 248 the reader will find our own views as to the occurrence and significance of reactions and of their causes as we understand them.

CHAPTER VII

PRACTICAL RESULTS IN PROPHYLACTIC VACCINATION

Proceeding now to the consideration of the benefits derived from our method of preventive vaccination against tuberculosis we intend to advert only to the results in those children and adults whom we had an opportunity of personally examining again after periods of a month or more, and who had reacted positively to the administration of the vaccine. The reexaminations were made at various periods ranging from one to forty-two months; for the sake of brevity, and in order to group the cases, it was necessary to include intervening periods in such a manner that the time of reexamination of the individual cases approached nearest the time stated in the tabulations.

GENERAL IMPROVEMENT. NUTRITION

The general improvement occurring after vaccination can be shown best by the gain in weight experienced by the vaccinated persons, as it appears manifest after a given time. In children the natural increase must of course be taken into consideration, for if the recorded gain in weight were not greater after vaccination than it was before, a claim of benefit from the treatment would not be tenable; neither can the influence of the treatment become manifest in this direction in those children who, at the time of their vaccination, were not materially below the average weight for their age.

The question then arises, What is the proper weight for a child at a given age-period, and how does this differ in children who have experienced a tuberculosis-infection?

Unfortunately it is not possible to speak of a *normal* weight scale for children, because no data are available by which such

a normal scale can be established. Since Quetelet,[1] in 1835, and Bowditch,[2] in 1877, published the results of their weighings of children, few attempts seem to have been made to control and revise their results which are still used as a basis for similar tabulations. Most of the investigations that have been recorded are not comparable because the materials have been handled from too greatly differing viewpoints, and many of them are not cited uniformly in the literature available to us, which renders the use of any but original publications unsatisfactory. While most authors have weighed their children without making allowance for the clothing (for the deduction of which different figures are, however, given), Schröder's[3] boys, for instance, were weighed naked. In Bowditch's material, which comprises 24,595 school children in Boston, anything like regularity and uniformity, e.g., with respect to the hour of the day, the state of bowels and bladder, etc., must have been impossible, and yet these and many other things may alter the results of weighing very materially. Another point of importance is that the figures given are irrespective of the state of health of the children and, in consequence, the results do not represent the normal, but the average figures for the respective age-periods. This is of particular importance for our inquiry because, since the first infection, which ultimately leads to phthisis, occurs in the majority of cases in childhood, it follows that in many children the infection had probably already produced certain nutritive disturbances which are reflected in the weight-behavior. On account of these and other considerations, the establishment of a normal weight-scale for children, and the readjustment of the existing average weight-scales, is urgently called for.

The available literature on the weight-behavior in pulmonary tuberculosis does not aid us much in our inquiry because it deals, with few exceptions, with adult patients ill with clinically manifest tuberculosis, while our children's material is largely made up of so-called "pretuberculous" subjects in the

[1] QUETELET; Sur l'homme et le développement de ses facultés. Paris, 1835. cf. VIERORDT, in GERHARDT; Handbuch d. Kinderkrankh.; Tübingen, I, 1881, 227.

[2] H. BOWDITCH; The Growth of Children; Boston, 1877. cf. VIERORDT, in GERHARDT, *loc. cit.*

[3] WM. SCHRÖDER; Archiv f. Hygiene. 1886, IV, 39.

truly early (or primary) stage of the disease which is designated by Hamburger, also by Petruschky, as the stage of lymph-gland involvement. In this stage the loss of weight that accompanies the "consumption" of the body is not yet active, and we do not have to deal with fever, night-sweats, hemoptysis, destructive processes attended by copious secretions, all of which are factors in the emaciation of phthisical persons. Among the other causes of loss of weight, some or all of which may be of import even in early and prophylactic cases, we may mention anorexia, dyspepsia and diarrhea, and especially the action of toxins. That the loss of weight may be a toxic symptom appears, according to C. Fischer[4] from the fact that, in intestinal tuberculosis, it is often out of all proportion to the slight extent of the intestinal and glandular lesions. This view is confirmed by an observation of Kuthy,[5] that guinea-pigs inoculated with large doses of sputum from a consumptive, which contained many bacilli but had been sterilized by moist heat, did not show tuberculous changes but perished of toxic marasmus; further, by a like experience of Wolff-Eisner (*ibidem*), who, after injecting ten guinea-pigs with a dead culture of tubercle bacilli, one year old, found them at autopsy in a state of severe cachexia, but free from tuberculosis.

We have ourselves made corresponding observations in a much larger number of animals, which died under treatment with fractions of the body-substances of tubercle bacilli in solution, especially with a pepton and with certain alcohol-extractives, in doses of only 0.1 milligram. Death followed after these doses in some instances within twenty-four hours, and when the animals had lived longer the autopsy results were the same as those described by Wolff-Eisner.

Such degrees of poisoning with products of the tubercle bacillus, in small animals like guinea-pigs, are not noted in man, unless we accept the marasmus and cachexia of the terminal stages of phthisis as being due to it alone. Nevertheless the loss of weight, while slower in occurrence and more mo
degree, is also an early symptom and is sufficient
to be properly considered in clinical diagnosis. Th

[4] C. FISCHER; Zeitschr. f. Tuberkulose. 1910, XVI, 33
[5] KUTHY & WOLFF-EISNER; Die Prognosestellung b. d. I
Berlin u. Wien, 1914, S. 183.

of the lost weight, after the administration of vaccine and of like products of the tubercle bacillus, is therefore significant and indicates the removal of the cause which stood in relation to the previous loss; and the cause of this loss is, in the last instance, the tuberculous infection which has evolved to a sufficient degree to manifest itself in this manner if by no other symptom.

Years ago, Kohlschütter [6] claimed diagnostic value for the weight-curve in tuberculous persons, and Wolff-Immermann [7] considered it as of equal import with other methods of examination and observation. At the Washington Tuberculosis Congress, Shoemaker [8] advocated monthly weighings of all school children, in order to separate the delicate from the robust, and to refer the former to the physician; for he claims that there is no clinical sign so reliable or so expeditiously ascertained as the decline of body-weight. All healthy children gain in weight, and failure to do so is an indication of something wrong. Miller and Woodruff [9] even believe that malnutrition is sometimes the only appreciable evidence of tuberculosis in children.

It should, of course, be kept in mind that a "normal" weight does not necessarily indicate a normal organization. Even in progressive tuberculosis a loss does not always take place, and Kohlschütter cites the case of a young man who gained almost four pounds in weight in the course of a fatal tuberculous pneumonia following upon a severe hemoptysis. Ordinarily, however, the unfavorable influence of tuberculosis upon the body as expressed by loss of weight is undoubted. In 274 "pre-tuberculous" and tuberculous children in the County of Cumberland, examined by Fraser,[10] there were, for their respective ages:

Above the average weight	68 or 24.8 per cent
Of average weight	30 or 10.9 " "
Below average weight	176 or 64.3 " "

[6] E. KOHLSCHÜTTER; Volkmann, Samml. klin. Vortr. No. 303, 1887.

[7] WOLFF-IMMERMANN; Münch. med. Woch. 1898, XLV, 777.

[8] C. H. SHOEMAKER; Trans. Washington Tuberculosis Congress. 1908, II, 598.

[9] J. A. MILLER and I. O. WOODRUFF; *ibidem*. 1908, II, 487.

[10] KENNETH FRASER; Brit. Journ. of Tuberculosis. 1915, IX, 1.

The degree of the loss in weight, which has been experienced, has also been considered as of prognostic importance. In a study of 8406 tuberculous patients over fifteen years old, de la Camp [11] observed the weight-behavior, comparing it with Landois' tables of "normal" weights. Those patients who weighed less than 70 per cent of their proper weight had an unfavorable prognosis. An admission-weight of 70 to 80 per cent of the normal gave a doubtful prognosis, but if the admission-weight was 80 per cent or more of the normal, the prognosis was favorable. Frankenau [12] notes that in several infants with symptoms of tuberculosis, who survived the first year of life, the weight was always relatively good, never less than 65 per cent of the normal at the time the diagnosis was established. Of those who died, only few showed as favorable weight-records as 65 per cent of the normal. He cites Engel to the effect that in babies in good nutrition the tuberculosis is usually limited to the lymph-glands and that death occurs only in consequence of accidents leading to dissemination; on the other hand, in poorly nourished babies the organs are likely to become involved in the tuberculous process, which then takes a rapidly fatal course.

In respect to these observations it is hardly necessary to point out that the previous loss in weight was the expression of the greater or less marked evolution of the tuberculous disease, and that the prognosis was necessarily better in those cases in which the absorption of poisonous bacterial products was slight or had not yet influenced the nutrition.

Ordinarily a gain in weight in tuberculous persons is taken as an index of improvement, and justly so, especially if it is accompanied by other evidences such as loss of fever, diminution of expectoration and of night-sweats, etc. Yet a gain in weight may merely mean improvement in physical health without a corresponding healing, and, reversely, a failure to gain weight does not always mean non-improvement. This is particularly true of those patients who have not suffered a great loss of weight and who are very close to their physiological standard; in them a gain cannot be expected to be as marked as in those who have much to make up. This is in accord with

[11] De la Camp; Berlin Tuberculosis Congress. 1899, p. 447.

[12] Arnold Frankenau; Inaug. Diss. Erlangen. 1912, S. 14.

the observation of Berger [13] in the Basel Sanatorium at Davos, that patients who are below weight and in poor nutrition gain faster than others, under otherwise like conditions, and it is also borne out by Julian in his observations that the children, who had been much below weight before, showed the greatest relative increase after vaccination.

Observations in a number of Danish tuberculosis sanatoria [14] indicate that the gain in weight experienced by the patients showed marked seasonal variations, in that considerable gains were recorded for the total of the patients during mild and humid periods, while the gains were small or even negative during cold and dry periods, as ascertained by weekly weighings. Consequently the summer half-year showed more "positive" weeks than the winter half-year, and the latter had nearly twice as many "negative" weeks as the former. The most decided average gains were recorded in June, July, August, September, while losses occurred, for the average of the patients, during October, November, December. This variability is explained by Strandgaard as standing in relation to meteorological conditions. Half-yearly weighings, for six years, of 253 schoolgirls, from three to sixteen years old, in Jaegerspris, Denmark,[15] showed an average increase of 1.9 pounds during the winter term and one of 3.0 pounds during the summer term.

In estimating the net gain experienced by tuberculous patients, it is to be remembered that the checking of further loss constitutes a net gain in itself. Further, in evaluating the gain obtained, THE MOST IMPORTANT CRITERION IS NOT SO MUCH THE ACTUAL PLUS OF TOTAL BODY-BULK WHICH HAS BEEN ATTAINED OVER THE LOWEST PREVIOUS FIGURE, BUT RATHER THE AMOUNT OF GAIN WHICH IS RETAINED AND PRESERVED PERMANENTLY. It is of importance to note that the children treated with vaccine were found on later examinations to have retained their gains in weight, and thereafter to have continued to gain in accordance with the average progress for their respective age-periods.

We have both age- and weight-records in 1092 of the 1512 chil-

[13] ARMAND BERGER; Inaug. Dissertation; Basel, 1905. Zeitschr. f. Tuberkulose; 1905, VII, 521.

[14] cf. N. J. STRANDGAARD; Beitr. z. Klin. d. Tuberkulose. 1914, XXXII, 179.

[15] M. VAHL, cited by STRANDGAARD; *loc. cit.*

dren under consideration and, with few exceptions, they belonged to the well-to-do class, which fact, according to Bowditch's figures, tends to raise the average weights. In tabulating the results in these children we find such large variations at a given age period that it is hardly possible to explain at least some of them otherwise than by the influence which the preceding tuberculous infection may or may not have exerted upon the general nutrition. Thus our weight determinations, before vaccination, varied within the following limits:

Age, Years.	Weight, lbs. Minimum.	Maximum.
2– 3	21	38
3– 4	21	36
4– 5	24	37
5– 6	30½	53
6– 7	29	46
7– 8	38½	56
8– 9	44½	62
9–10	42	54
10–11	42	62
11–12	50	72
12–13	60½	90
13–14	66	105

An attempt to compare these weights with the old tables of Bowditch and of Quetelet affords little or no light in regard to what should be considered as normal, because neither of them could separate those children who had suffered a tuberculosis-infection from those who were free from it. Bowditch mentions one factor which may alter the average weight figures very materially, viz., the economic condition of the parents of his children. His maxima and minima appear to vary as greatly as do our own. Quetelet's investigations confined themselves to comparatively small numbers of children (10 for each age-period and group), and in his selection he was probably better able to eliminate abnormals. His increases in weight from year to year are accordingly more uniform.

For the convenience of those who do not have access to these tables, we reproduce those of Quetelet and of Bowditch, as they are cited by Vierordt; also the table used by the Metropolitan Life Insurance Company, which is taken from Wellcome's *Excerpta Therapeutica,* and for which we are indebted to the

courtesy of the Chief Medical Director, Dr. Knight; we also give the table of Fleischmann, of monthly average weights during the first year of life, likewise taken from Vierordt. We have converted the kilograms into pounds, calculating one kilogram as being equal to 2.2 pounds.

AVERAGE WEIGHTS OF CHILDREN IN POUNDS

Age	Quetelet		Bowditch		Metropolitan Life Ins. Co.		Influence of Economic Condition, Bowditch			
							Boys		Girls	
	Boys	Girls	Boys	Girls	Boys	Girls	Well-off	Poor	Well-off	Poor
0	7.04	6.40								
1	20.79	19.34			18.5	18.0				
2	24.95	23.47			32.5	25.25				
3	27.43	25.94			34.0	31.5				
4	31.31	28.6			37.0	36.0				
5	34.69	31.59			40.0	39.0				
5–6			41.01	39.58			41.14	40.92	40.46	39.40
6	37.93	35.2			44.5	41.75				
6–7			45.08	43.19			45.41	44.97	44.04	43.03
7	42.02	38.57			49.75	47.5				
7–8			48.97	46.42			49.65	48.82	47.81	47.06
8	45.67	41.98			55.0	52.0				
8–9			53.81	51.57			54.52	53.55	52.67	51.70
9	49.83	46.99			60.5	55.5				
9–10			59.11	57.00			59.75	59.09	58.65	56.63
10	53.94	51.74			67.5	62.0				
10–11			65.16	62.24			66.18	65.08	63.62	61.84
11	59.62	56.43			72.0	68.0				
11–12			70.05	68.71			71.65	69.52	70.33	67.87
12	65.60	65.60			76.75	76.5				
12–13			76.76	78.17			80.21	75.72	80.01	77.35
13	75.64	72.47			82.5	87.0				
13–14			84.68	88.46			88.40	83.23	90.49	69.65
14	85.07	80.74			92.0	96.75				
14–15			94.49	98.23						
15	95.96	88.81			102.75	106.25				
15–16			106.90	105.86						
16	109.27	95.85			119.0	113.0				

INCREASE IN WEIGHT DURING FIRST 12 MONTHS OF LIFE (Fleischmann)

Age Months	Weight lbs.
0	7.7
1	10.0
2	11.0
3	14.0
4	15.4
5	16.6
6	17.5
7	18.3
8	19.0
9	19.6
10	20.24
11	20.79
12	21.0

Taking the total of our own 1092 children, in whom the age and weight are recorded, we find that, before their vaccination and in round numbers, forty per cent of our children were more or less under weight; forty-five per cent were apparently of average weight, and fifteen per cent exceeded the average weight for their respective age-periods.

In 20 infants giving positive reactions to tuberculin or to vaccine we find, on comparison with Fleischmann's table for the weight of nurslings from month to month, that 13, or 65 per cent, were under weight; 5, or 25 per cent, were of average weight, and 2, or 10 per cent, were above average weight.

According to Quetelet, the normal increase in weight is about four pounds per year up to the age of ten years; six pounds for the eleventh and twelfth years, and ten pounds for the thirteenth and fourteenth years of life. It seems to us then that, if we accept as a normal increase one-half pound per month in children under twelve years, and one pound per month in children over twelve years of age, deducting that amount from the weight shown on reexamination, we have a conservative criterion for practical purposes in the estimation of the gain in weight experienced by our children, over and above that which could be expected to have occurred under ordinary circumstances. Although it would have been desirable for the sake of entire accuracy to take into consideration other factors, among them the sex, height, the bony frame, the differences in clothing in boys and girls and the weight at different seasons, the advantages enjoyed by children in well-to-do environments, the fact that after periods of from one to three and one-half years some of those classed as children had actually become adults, etc., we are unable to do better, and leave the reader to judge for himself in how far we may have approached a conservative estimate of the results.

The following tabulation gives the average gains of 279 vaccinated children and adults, determined at periods of from one to forty-two months after vaccination (p. 186).

The increase in weight shown in this tabulation would appear still greater if we were to consider and tabulate only those individuals in whom we had found less than the average weight before vaccination, the maximal gains observed having been, as a rule, most when the previous deficiency was greatest. In

GAIN IN WEIGHT AFTER VACCINATION

Time After Vaccination	No. of Persons	No Gain or Loss	Gained lbs.	Minimum lbs.	Maximum lbs.	Average lbs.	Deduct Average	Excess Over Average	Per cent of Excess	Remarks
23 Infants, 3—22 Months										
1 month	13	1	12	1.5	6	3.3	0.5	2.8	84.8	Child who failed to gain, lost 3 lbs. by fever-reaction to 103° F.
2 months	3	—	3	4.0	6.5	5	1	4	80	
3 "	1	—	1	10	10	10	1.5	8.5	85	
6 "	2	—	2	4	12.5	3.2	3	5.2	16.2	
12 "	2	—	2	8	11	9.5	6	3.5	36.7	
18 "	1	—	1	10	10	10	9	1	10	
36 "	1	—	1	....	20	—	18	2	....	
222 Children, 2—16 Years										
1 month	54	2	52	1	10	3.6	0.5	3.1	36	One of 2 children with no gain had fever-reaction, 102° F.
2 months	27	—	27	2	13	5.9	1	4.9	83	
3 "	14	—	14	2	12	6.5	1.5	5	77	
6 "	18	—	18	2.5	26	9	3	6	66.6	
9 "	19	—	19	5	24	11.3	4.5	6.8	60	
12 "	12	—	12	6	27	14.1	6	8.1	57.4	
18 "	23	—	23	9.5	21	14	9	5	35.7	
24 "	14	—	14	9	30.5	16.3	12	4.3	26.4	
30 "	10	—	10	9.5	27	16	15	1	6.2	
36 "	13	—	13	15.5	45.5	25.6	18	7.6	29.7	Some have reached adult age.
42 "	18	—	18	14	30.5	21	21	—	—	
34 Adults, 17—60 Years										
1 month	18	2	16	3.5	20	6.3	—	....	....	
2 months	2	—	2	7.5	7.5	7.5	—	....	....	
3 "	3	—	3	6	30	11.6	—	....	....	
6 "	6	—	6	6	21	12.3	—	....	....	
9 "	3	—	3	10	27	16	—	....	....	
12 "	1	—	1	....	15	15	—	....	....	
42 "	1	—	1	....	22	22	—	....	....	

adults the increase in weight is not subject to deductions based upon the weight-gain on account of growth, and therefore the total gain can be claimed to stand in relation to the vaccination. A number of our adult patients were decidedly below normal weight before vaccination and, according to their statements, the weight had, in most of them, never exceeded that materially which we found on the occasion of our first examination. The comparative increase of weight in both children and adults, accordingly as this had been normal or decidedly below normal, will be seen further in a number of cases which are described below with more detail; we have added at the same time our findings in specific antibodies and the results of animal experiments, when they were made, with the sera of the vaccinated individuals. These cases are selected in so far as two cases are given for each period after vaccination, one showing the minimal and the other the maximal gain in weight.

REEXAMINED AFTER THREE MONTHS

CASE No. 1. "Normal" weight. Gained 1½ pounds.

Jan. 5, 1912. M. R., male, age 8½ years; mother has tuberculosis; appearance fair; weight 50 lbs.; temperature normal; respiration normal; lungs normal; no enlarged glands; v. Pirquet, marked reaction.

Serum examination: Agglutinins 1:5; Precipitins 1:400; Amboceptor trace with tubercle bacilli; other antigens negative; Bacteriolysis negative; Blood-alkalinity 0.90.

Jan. 8, 1912. Vaccine 1 mgr.; marked local reaction.

Jan. 18, 1912. Vaccine 3 mgr.; marked local reaction.

April 1, 1912. Reexamination. Appearance fair; weight 51½ lbs.; Feels well.

Serum examination: Agglutinins 1:75; Precipitins 1:800; Amboceptor 1:8 or plus with all antigens. Bacteriolysis 2; Blood-alkalinity 1.1.

Sept. 27, 1912. Serum examination: Agglutinins 1:100; Precipitins 1:200; Amboceptor 8—4—8—20. Bacteriolysis 2.

Guinea-pig No. 145. Infected with 0.01 mgr. of virulent tubercle bacilli, incubated for 24 hours with the above immune serum *; killed after 108 days. Autopsy: normal.

* In this and the following animal experiments, the tubercle bacilli (0.01 mgr.) were always incubated with the same quantity of immune or of normal serum, or of normal salt solution. Further details of the autopsies will be found in the several tables in Chapter XII.

Guinea-pig No. 146. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 58 days. Autopsy: generalized tuberculosis.

CASE No. 2. Below "normal" weight. Gained 10 pounds.

B. M., male, age 10 months; mother has tuberculosis; appearance pale; weight 16½ lbs.; pulse 90; lungs normal; both cervical groups of lymph-glands enlarged and numerous; v. Pirquet marked reaction.

Serum examination: Agglutinins 1:5; Precipitins 1:400; Amboceptor negative; Bacteriolysis negative; Blood-alkalinity 0.9.

Guinea-pig No. 167. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 73 days, of generalized tuberculosis.

Aug. 7, 1912. Vaccine 1 mgr.; marked local and general reaction; temperature 102° Fahrenheit.

Aug. 12, 1912. Serum examination: Agglutinins 1:30; Precipitins 1:400; Amboceptor 1:4 with all antigens; Bacteriolysis 1 plus; Blood-alkalinity 1.1; weight 19 pounds.

Guinea-pig No. 175. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with 0.2 cc. of the above serum; killed after 169 days. Autopsy: no tuberculosis.

Guinea-pig No. 172. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 33 days. Autopsy: genuine and pseudo-tuberculosis.

Nov. 1, 1912. Reexamined. Appearance good; weight 26½ lbs.; cervical lymph-glands not palpable.

Serum examination: Agglutinins 1:50; Precipitins 1:200; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 1 plus; Blood-alkalinity 1.2.

REEXAMINED AFTER SIX MONTHS

CASE No. 3. "Normal" weight. Gained 3 pounds.

Oct. 23, 1911. M. H., female; 6 years old; father has tuberculosis; appearance fair; weight 39½ lbs.; temperature normal; pulse 84; lungs normal; right cervical group of lymph-glands enlarged; v. Pirquet marked.

Serum examination: Agglutinins 1:5; Precipitins 1:1600; Amboceptor negative; Bacteriolysis negative; Blood-alkalinity 1.0.

Oct. 27, 1911. Vaccine 2 mgr. Local reaction marked; temperature 99° Fahrenheit.

April 12, 1912. Appearance fair; weight 42½ lbs.; temperature normal; pulse 88.

Serum examination: Agglutinins 1:50; Precipitins 1:400; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 1 plus; Blood-alkalinity 1.0.

Guinea-pig No. 524. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 67 days. Autopsy showed no cause; no tuberculosis.

Guinea-pig No. 558. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal serum. Died after 55 days. Autopsy: generalized tuberculosis.

CASE No. 4. Below "normal" weight. Gained 15 pounds.

Oct. 27, 1912. A. B., female; 11 years old; father has tuberculosis; appearance rather thin; weight 65 lbs.; temperature normal; pulse 88; both cervical and right axillary groups of lymph-glands enlarged; lungs, slight percussion and auscultation signs in right upper lobe below clavicle; v. Pirquet marked reaction.

Oct. 27, 1912. Serum examination: Agglutinins 1:20; Precipitins 1:800; Amboceptor negative; Bacteriolysis negative; Opsonic Index 0.8; Blood-alkalinity 1.0.

Oct. 28, 1912. Serum examination: Agglutinins 1:20; Precipitins 1:200; Amboceptor negative; Opsonic Index 1.0; Blood-alkalinity 1.0.

Guinea-pig No. 377. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 107 days, of generalized tuberculosis (fibrous changes are a marked feature).

Nov. 5, 1912. Vaccine 2 mgr. Local reaction marked; temperature 100° Fahrenheit.

April 17, 1913. Reexamination. Appearance good; weight 80 lbs.; temperature normal; pulse 83; cervical and axillary lymph-glands not palpable; lungs normal.

Serum examination: Agglutinins 1:100; Precipitins 1:400; Amboceptor 1:8 with all antigens; Bacteriolysis 1½; Blood-alkalinity 1.2.

Guinea-pig No. 560. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 95 days. Autopsy showed no cause; no tuberculosis.

Guinea-pig No. 559. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 125 days, of generalized tuberculosis.

REEXAMINED AFTER NINE MONTHS

CASE No. 5. "Normal" weight. Gained 2¼ pounds.

March 23, 1914. J. S., male; age 3 years; no known exposure; appearance good; weight 35¼ lbs.; temperature normal; pulse 90; lungs normal; lymph-glands not palpable; v. Pirquet positive, moderate reaction.

Serum examination: Agglutinins 1:20; Precipitins 1:200; Amboceptor negative; Bacteriolysis negative; Blood-alkalinity 0.95.

March 23, 1914. Guinea-pig No. 2354. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 45 days, of acute miliary tuberculosis.

Guinea-pig No. 2355. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 45 days, of acute miliary tuberculosis.

March 25, 1914. Vaccine 1.5 mgr.; marked local reaction.

April 1, 1914. Serum examination: Agglutinins 1:60; Precipitins 1:400; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 1 plus; Blood-alkalinity 1.2.

April 3, 1914. Guinea-pig No. 2358. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Killed after 52 days; no tuberculosis.

Guinea-pig No. 2368. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal rabbit serum. Killed after 52 days; has generalized tuberculosis.

Sept. 4, 1914. Reexamination: appearance good; weight 37½ lbs.; temperature normal; lungs normal; lymph-glands not palpable.

CASE No. 6. Below "normal" weight. Gained 32 pounds.

June 4, 1913. E. T., female; age 8 years; mother died of tuberculosis; sister has tuberculosis; appearance pale; weight 50 lbs.; temperature normal; pulse 90; v. Pirquet marked reaction; lymph-glands, right cervical enlarged; lungs, doubtful circumscribed area in right apex.

June 4, 1913. Serum examination: Agglutinins 1:25; Precipitins 1:400; Amboceptor traces; Bacteriolysis trace; Blood-alkalinity 0.7.

Guinea-pig No. 602B. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 153 days. Autopsy: chronic tuberculosis; marked fibrosis.

Guinea-pig No. 606B. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 56 days. Autopsy: acute miliary tuberculosis.

June 21, 1913. Vaccine 2 mgr.; local reaction marked; temperature 99.5° Fahrenheit.

Sept. 29, 1913. Reexamination: appearance good; weight 62 lbs.; several hard palpable glands, right cervical; lungs negative.

Sept. 29, 1913. Serum examination: Agglutinins 1:75; Precipitins 1:200; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 2; Blood-alkalinity 1.1.

Guinea-pig No. 907. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum; killed after 114 days; no tuberculosis.

Guinea-pig No. 906. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 112 days; generalized tuberculosis.

March 15, 1914. Reexamination: appearance good; weight 82 lbs; no glands palpable; lungs normal.

Serum examination: Agglutinins 1:100; Precipitins 1:400; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 2; Blood-alkalinity 1.15.

Guinea-pig No. 2287. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 23 days, of marasmus; no tuberculosis.

Guinea-pig No. 2291. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal rabbit serum. Died after 38 days; acute miliary tuberculosis.

Guinea-pig No. 2292. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 39 days; genuine and pseudo-tuberculosis.

Rabbit No. 336. Infected with 0.01 mgr. of virulent bovine tubercle bacilli incubated for 24 hours with the above serum. Killed after 71 days; no tuberculosis.

Rabbit No. 347. Control. Infected with 0.01 mgr. of virulent bovine tubercle bacilli incubated for 24 hours with normal rabbit serum. Killed after 71 days; generalized tuberculosis.

Rabbit No. 348. Control. Infected with 0.01 mgr. of virulent bovine tubercle bacilli incubated for 24 hours with normal salt solution. Killed after 71 days; generalized tuberculosis.

REEXAMINED AFTER TWELVE MONTHS

CASE No. 7. "Normal" weight. Gained 7 pounds.

June 16, 1912. C. F., female; age 13 years; brother tuberculous, treated successfully three years ago; appearance good; weight 100 lbs.; temperature normal; pulse 88; lungs, slight physical signs and retraction of right apex; no glands.

Serum examination: Agglutinins 1:20; Precipitins 1:200; Amboceptor trace; Bacteriolysis negative; Blood-alkalinity 1.0.

Guinea-pig No. 61. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 104 days; generalized tuberculosis.

June 19, 1912. Vaccine 4 mgr.; marked local reaction; temperature 99.5° F.; nausea.

June 24, 1912. Serum examination: Agglutinins 1:60; Precipitins 1:400; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 3; Blood-alkalinity 1.15.

Guinea-pig No. 67. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 86 days, of accident; no tuberculosis.

Guinea-pig No. 73B. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal salt solution. Died after 73 days; generalized tuberculosis.

June 4, 1913. Reexamination: appearance good; weight 107 lbs.; temperature normal; pulse 72; lungs, no change; no glands palpable.

Serum examination: Agglutinins 1:100 plus; Precipitins 1:800; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 2 plus; Blood-alkalinity 1.2; Tuberculin test negative.

CASE No. 8. Below "normal" weight. Gained 14 pounds.

Sept. 5, 1912. D. E. S., female; age 3 years; father has tuberculosis; appearance poor; weight 20½ lbs.; temperature normal; pulse normal; lungs normal; left axillary and both inguinal groups of lymph-glands enlarged; v. Pirquet moderate reaction.

Sept. 9, 1912. Serum examination: Agglutinins 1:10; Precipitins 1:400; Amboceptor negative; Bacteriolysis negative; Blood-alkalinity 0.9.

Guinea-pig No. 236. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 127 days; generalized tuberculosis.

Vaccine 0.5 mgr.; moderate local reaction; temperature normal.

Sept. 16, 1912. Vaccine 1.0 mgr.; marked local reaction; temperature 99° Fahrenheit.

Sept. 30, 1912. Serum examination: Agglutinins 1:100; Precipitins 1:400; Amboceptor 1:8 with tubercle bacilli; Bacteriolysis 2; Blood-alkalinity 1.1.

Guinea-pig No. 277A. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Killed after 173 days; no tuberculosis.

Guinea-pig No. 279. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal human serum. Died after 84 days; generalized tuberculosis.

Aug. 26, 1913. Reexamination: appearance good; weight 34½ lbs.; lungs normal; one hard gland, pea-size, palpable in left axilla.

REEXAMINED AFTER TWO YEARS (ADULTS)

Case No. 9. Normal weight. Gained 3 pounds.

Sept. 20, 1911. Miss H. L.; age 19 years; tuberculosis in family; appearance good; weight 130 lbs.; temperature normal; pulse 78; no glands; lungs, slight physical signs in right upper lobe.

Serum examination: Agglutinins 1:30; Precipitins 1:200; Amboceptor negative; Blood-alkalinity 0.9.

Guinea-pig No. 246. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 38 days; acute miliary tuberculosis.

Guinea-pig No. 247. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal guinea-pig serum. Died after 35 days; acute miliary tuberculosis.

Vaccine, 5 mgr.; marked local reaction; temperature 100° F.; focal reaction in right lung.

Sept. 28, 1911. Reexamined. Serum examination: Agglutinins 1:60; Precipitins 1:400; Amboceptor 1:8 with all antigens; Bacteriolysis 3; Blood-alkalinity 1.15.

Guinea-pig No. 273. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Killed after 305 days; no tuberculosis.

Guinea-pig No. 266. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal human serum. Died after 94 days; generalized tuberculosis.

Oct. 1, 1914. Reexamination: in good health; weight 133 lbs.; lungs normal; no further record.

CASE No. 10. Below normal weight. Gained 28 pounds.

Miss H. M.; age 19 years; father has tuberculosis; appearance, sallow color; thin; weight 98 lbs.; temperature 100° F.; pulse 108; lungs, physical signs, right apex posteriorly; right cervicals, several enlarged glands.

Serum examination: Agglutinins 1:15; Precipitins 1:200; Amboceptor traces; Bacteriolysis negative; Blood-alkalinity 0.9.

Sept. 10, 1911. Vaccine 5 mgr.; local and general reaction; focal reaction in right upper lobe.

Sept. 18, 1911. Vaccine 10 mgr.; moderate local reaction.

Sept. 30, 1911. Serum examination: Agglutinins 1:125; Precipitins 1:200 Amboceptor 1:20; Bacteriolysis 2 plus; Blood-alkalinity 1.1.

April 12, 1913. Reexamination: appearance improved; weight 111 lbs.; lungs normal; one small hard gland, right cervical; temperature normal.

Serum examination: Agglutinins 1:150; Precipitins 1:400; Amboceptor 1:4 and 1:8 with all antigens; Bacteriolysis 2; Blood-alkalinity 1.05.

Guinea-pig No. 546. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with the above serum. Died after 194 days, of intestinal obstruction; no tuberculosis.

Guinea-pig No. 553. Control. Infected with 0.01 mgr. of virulent tubercle bacilli incubated for 24 hours with normal human serum. Died after 137 days; generalized tuberculosis.

Oct. 4, 1913. Reexamination: appearance good; weight 126 lbs.; lungs normal; one hard cervical gland.

Oct. 12, 1914. Reexamination: appearance good; weight 125 lbs.; lungs normal; cervical gland not found.

ENLARGED LYMPH-GLANDS

Comparatively few of the children who reacted positively to the vaccine did not also show enlargement of lymph-glands. Often these were small, and sometimes difficult to find, requiring careful palpation. The cervical groups were involved most frequently, appearing often in chains or groups; in a large number of instances the axillary or inguinal groups were enlarged likewise. It was not possible in our examinations to make the records so accurate as to enable us later to make perfectly reliable comparisons, nor is there any certainty that the glands were actually tuberculous, except in a small number of cases in which they became tender and enlarged after a dose of vaccine. We are thus able to consider statistically only those cases in which no enlarged glands could be found on reexamination, or those cases in which they were missed in certain groups, when they had been recorded as present on the

first examination, or again, when the record was sufficiently explicit in regard to their size and number in a particular group.

The importance which we attribute to the enlargement of lymph-glands in children, especially the cervical and axillary groups, in conjunction with other symptoms suggestive of tuberculous infection, is not conceded generally. Landis [16] says that, in childhood, all of the lymphoid tissue is in excess, and he justly criticizes the attempts of some observers to base a diagnosis of tuberculosis on no other grounds than the one that the cervical lymph-nodes are palpable. He has found the superficial lymph-nodes readily palpable in practically all children from the second to the eleventh or twelfth year, irrespective of their social condition.

Miller & Woodruff [17] conclude from the results of an examination of 150 children of tuberculous parents that the evidence for enlargement of the cervical lymph-nodes, as a determining factor in the diagnosis of tuberculosis, is not conclusive. In any event, they assert, it does not seem to have an influence sufficiently consistent to make it of aid in the diagnosis of tuberculosis in a doubtful case. Wright [18] points out that it must not be taken for granted that all glandular enlargements, in children who are likely to be subject to tuberculosis, are necessarily tuberculous; they may be merely inflammatory or due to other causes. Osler [19] has observed, in the post-mortem room, enlargement of the cervical, axillary and inguinal glands, without evidence of tuberculosis.

All this is very true, and we are far from asserting that every enlarged lymph-gland is tuberculous, or that it indicates the presence of active tuberculosis in a child, any more than a positive v. Pirquet reaction does so. The significance of enlarged lymph-glands is not so much in the indication of actual, as rather of possible tuberculosis, in the indication of an infection with tubercle bacilli which may be latent, or may not even have produced any manifest tuberculous lesions, but which consti-

[16] H. R. M. Landis; Therap. Gazette; 1915, XXXIX, 77.

[17] J. A. Miller & I. O. Woodruff; *loc. cit.* (p. 180).

[18] G. A. Wright, in Kelynack; Tuberculosis in Infancy and Childhood. New York, 1908, p. 160.

[19] Wm. Osler, in Kelynack; *loc. cit.*, p. 18.

tutes the possible origin of later disease. In some feeding-experiments with young guinea-pigs, Julius Bartel [20] found the infection to be followed by a period of latency, during which no specific histological tuberculous changes could be discovered, although tubercle bacilli were demonstrated in the lymph-glands. In the affected animals, the author observed emaciation, retardation of growth, *occasionally a swelling of lymph-glands, not characteristic of tubercle;* in short, he found a symptom-complex such as it is frequently met with in children of tuberculous families, who are not subjects of active tuberculosis, but who present indubitable signs of having experienced an infection with tubercle bacilli. If it is considered that in one of Bartel's guinea-pigs this latency of tubercle bacilli, with absence of histologically specific tuberculous changes, was observed to obtain 104 days after a single exposure to infection, and if the brief span of life of the guinea-pig is compared with the much longer one allotted to humans, the conclusion is justified that, even allowing for differences in the species-resistance to tubercle bacilli, of humans and of guinea-pigs, one must assume a stage of infection with tubercle bacilli, preceding manifest tuberculous changes, in which the symptoms are general, not specifically tuberculous, and one must admit that this stage may last for a considerable period of time. Such a conclusion is in full accord with the views of Petruschky and of Hamburger, both of whom postulate a primary stage of tuberculosis, in analogy to the primary stage of syphilis, which they call the *stage of lymph-gland infection* or involvement. This primary stage cannot be diagnosed, in many cases, as one of actual tuberculous disease; but it is the stage during which prophylactic endeavor promises the best results, and during which preventive vaccination should be done preferably. As Engel points out, while adults usually remain free from tuberculous disease after they have once overcome the first infection ("apex-focus"), children are not safe from dissemination of tuberculosis, no matter how small the tuberculous foci in their organs may be. The presence of enlarged lymph-glands, therefore, usually permits, and even justifies the presumptive diagnosis of tuberculous infection if not of tuberculous disease, and consequently presents an indication for protective immunization.

[20] JULIUS BARTEL; Wien. klin. Woch. 1905, XVIII, 155.

Our comparative results show that ten per cent of the children in whom enlarged lymph-glands had been found on first examination had none after one month, and that this percentage increased as the periods between vaccination and reexamination became longer.

In 110 reexaminations made after from one month to and including four months, no apparent change was recorded in 28, or 25.4 per cent; there was a diminution of glands, in comparison with previously involved groups or numbers, in 49, or 44.5 per cent; and no glands were palpable in 33, or 30 per cent, of the cases.

On reexaminations of children, made between five and eighteen months after vaccination, no change was found in the number or size of glands in 5, or 7.3 per cent; improvement had occurred in 28, or 41.1 per cent, and no glands were palpable in 35, or 51.1 per cent. For the subsequent periods, up to three and one-half years, there are 48 children, with improvement recorded in 16, or 33.3 per cent, and disappearance of glands in 33, or 66.6 per cent of the number. Persisting enlargements were recorded frequently as being hard and shotty.

It should be mentioned also that there was a distinct fall in the frequency with which enlarged lymph-glands were noted on first examinations, after the twelfth year, suggesting that in the younger children in whom enlarged glands were found these would probably have disappeared spontaneously as the children grew older. This lower frequency of enlarged lymph-glands in older children can, in our opinion, not be used as an argument against any importance that might be attributable to enlargement of lymph-glands as a sign of infection with the tubercle bacillus. It must probably be referred to the gradual development of a specific resistance to an existing infection, by which this is overcome in the majority of cases, and which is usually associated with a local immunity. As long, however, as this local immunity is not developed, tubercle bacilli that may be present in enlarged lymph-glands are capable of determining pathological processes in the event of exciting causes becoming active, and for this reason the importance of enlarged lymph-glands as a symptom of a probable infection should not be underrated.

It must be assumed that a spontaneous diminution in en-

larged lymph-glands occurs in many cases in which the enlargement constituted a tissue-reaction to infection with tubercle bacilli. Owing to favorable conditions of environment, the infection is prevented from progressing into disease, and if the tubercle bacilli in the lymph-glands are of slight virulence, or if they die and become disintegrated, their removal by absorption does away with the irritation, so that a *restitutio ad integrum* becomes possible. This idea does not contradict the dominant views about phthisiogenesis; it is rather in their support.

In summing up the results of our observations, we may say that the enlarged lymph-glands were found to have disappeared, on reexamination, in approximately one-half of the cases in children, and that a distinct improvement was noted in a number of the others, although some of the glands were found to have persisted. They were then usually small and hard in consistency. The question of the tuberculous or non-tuberculous nature of the glands must, of course, be left open.

The records of 34 adults show that, on first examination, there were only five who had enlarged lymph-glands, and none were found on reexaminations made after nine months and up to three and one-half years.

PHYSICAL SIGNS IN THE CHEST

Physical signs in the chest were recorded, on examination before vaccination, in 545 out of a total of 1618 cases, or in 33.68 per cent, including all ages. Reexaminations could be made in 109 of these cases in the course of from one to forty-two months after vaccination. Our examination records being full and explicit, an exact comparison is possible, and we believe that by adopting the classification used by Dr. Julian [21] we can convey the results of our findings sufficiently without describing each case in detail. In explanation of our tabulation of the results, we may state that under "stationary" we include all findings in which, on reexamination, there was no decided change apparent, either on percussion or on auscultation, and that in a number of these cases the lesions found were

[21] C. A. JULIAN; *loc. cit.* (p. 138).

of such a nature that they had been recorded as probably healed or obsolete on the first examination before vaccination. "Stationary" therefore does not mean non-improvement. Under "improved" we include all those cases in which the lung-area, which gave abnormal physical signs on the first examination, was markedly diminished in extent, the remaining physical signs showing retrogression in that the percussion and auscultatory signs suggested healing and fibrosis as taking place. As "clear" we designate cases in which the physical signs had disappeared entirely, and as "healed lesions" those in which only strictly circumscribed areas of retraction could be found on reexamination, with a more or less short percussion-note and lack of vesicular quality.

In connection with the physical signs found in the lungs of children, it appears from our records that the localization of these signs does not differ from that which is found in adults; the physical signs were always found in the upper lobes and, as a rule, in the apices, the extension being seemingly downward.

The frequency with which we found an involvement in the lungs of the children whom we examined, is contrary to the experience of Bauer & Engel,[22] who consider lymph-gland and bone and joint tuberculosis to be characteristic of the disease in childhood, and assert that lung involvement is much less frequent. Other authors, however, have come to conclusions like ours. According to R. A. Young,[23] chronic pulmonary tuberculosis is common after the age of six years, and its physical signs follow on similar lines to those of adults. Franz Hamburger [24] says that apex-lesions are frequently met with after the sixth or seventh year of life and that they heal almost always in young children; after the tenth year they develop occasionally into progressive chronic tuberculosis as it is seen in adults. Bandelier & Röpke [25] call pulmonary tuberculosis fairly frequent in children over ten years of age, in whom it starts usually

[22] Bauer & Engel; Tuberkulose im Kindesalter. Würzburg, 1909, S. 263.

[23] R. A. Young, in Kelynack; Tuberculosis in Infancy and Childhood. New York, 1908, p. 86.

[24] Franz Hamburger; Allgemeine Pathologie und Diagnostik der Kindertuberkulose. Leipzig & Wien, 1910, S. 91-92.

[25] Bandelier & Röpke; *loc. cit.*, S. 443 (p. 152).

PHYSICAL SIGNS IN CHEST*

Before Vaccination	Physical Signs Slight, One Side Only					Physical Signs Slight, Both Sides					Physical Signs Marked, One Side Only					Phys. Signs Slight, One Side, Marked on Other Side					Physical Signs Marked on Both Sides				
	Results					Results					Results					Results					Results				
Examination After Months	Cases	Station.	Impr.	Clear	Healed	Cases	Station.	Impr.	Clear	Healed	Cases	Station.	Impr.	Clear	Healed	Cases	Station.	Impr.	Clear	Healed	Cases	Station.	Impr.	Clear	Healed
1	3	0	1	2	0	5	1	2	2	0						4	1	3	0	0					
	3	*1*	0	*3*	0	*6*	0	0	*5*†	0											*3*	0	*3*	0	0
2	3	0	0	3	0	6	0	2	4	0	1	0	1	0	0	3	0	3	0	0	2	0	2	0	0
						8	0	0	*8*	0															
3–4	4	0	0	4	0	6	0	0	6	0	0	0	0	0	0	1			1		1	0	1	0	0
																					4	0	1	2	1
5–6	1	0	0	1	0	5	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	*1*	0	0	0											*1*					*1*	0	0	*1*	0
6–9	2	0	0	2	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0	*3*	0	0	*2*	*1*
9–12	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2	0	0	2	0	1	0	0	1	0
																					1	0	0	0	*1*
12–18	2	0	0	2	0	2	0	0	1	1	0	0	0	0	0	3	0	0	2	1	2	0	0	0	2
18–24	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1
24–30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
																					1	0	0	0	*1*
30–36	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36–48	1	0	0	1	0	4	0	0	4	0	1	0	0	0	1	3	0	0	2	1	4	0	0	0	4
	1	0	0	*1*	0																*1*	0	0	*1*	0
Totals.....	23	2	1	20	0	38	1	4	32	1	3	0	1	0	2	18	1	6	9	2	27	0	7	7	13

* Mem. The italic figures indicate adults.

The reexaminations refer to the latest ones recorded.

"Stationary," no decided change from first findings.

"Improved," involved area smaller, physical signs less.

"Clear," no percussion or auscultation changes found.

"Healed," area small and circumscribed, as a rule with retraction and continuance of short percussion note, harsh or bronchial respiration.

In this tabulation 4 cases are not included in which no physical signs were noted on first examination and reexamination showed subclavicular or supraclavicular retraction with short percussion note and harsh respiration over the retracted areas—2 cases, 3½ years; 1 case, 18 months; 1 case, 9 months after vaccination.

† One case of interest in that the physical signs cleared up completely after an unmistakable focal and general reaction to an intravenous dose of vaccine. On examination 5 days after reaction, lungs were entirely clear.

in the apices and extends downward. In smaller children, the lung involvement is commonly secondary to bronchial gland tuberculosis and then appears first in the lower lobe; in fact, Bandelier & Röpke have often found the apices free from disease in these cases.

The localization of the tuberculous lesions in the lungs is confirmed by autopsy results, and its great frequency was first pointed out by Kuess.[26] More recently Emmett Holt [27] found in an analysis of 119 autopsies on tuberculous children that the lungs were implicated in 99 per cent. In 1060 autopsies on tuberculous children who died in hospitals in Vienna, H. Albrecht [28] found the lungs to be involved in all but seven, that is, in over 99 per cent. Ghon [29] reports on 170 cases of lung involvement in a total of 184 autopsies on tuberculous children.

Clinically, our frequency of apex localization is confirmed by Fraser's [30] examinations in 283 children which gave the following results:

Lesion	No.	Per cent
Right apex only	38	13.2
Left apex only	13	4.5
Both apices	169	58.8
Both apices and one or more foci elsewhere	52	18.0
Left apex; one or more foci elsewhere; not at right apex	8	2.6
Right apex; one or more foci elsewhere; not at left apex	6	2.0
Neither apex involved	1	0.3

On the other hand, the lower lobes of the lungs were found implicated with greater frequency in infants in the examinations of 73 cases of pulmonary tuberculosis and of 36 cases of meningeal tuberculosis with lung involvement, in patients in the New York Babies' Hospital, which were reported by

[26] GEORGES KUESS; De L'Hérédité parasitaire de la Tuberculose Humaine. Paris, 1898.

[27] EMMETT HOLT; Diseases of Infancy and Childhood. New York, 4th ed., 1907, p. 1076; cf. R. A. YOUNG, *loc. cit.*

[28] H. ALBRECHT; Wien. klin. Woch. 1909, XXII, 327.

[29] ANTON GHON; Der Primäre Lungenherd bei de Tuberkulose der Kinder. Berlin & Wien, 1912.

[30] KENNETH FRASER; *loc. cit.* (p. 180).

La Fetra [21] In these infants the location of the consolidations or of the cavities was anywhere from the apex to the lower lobe of either lung; involvement of the right lung, especially the upper and middle lobes, was twice as frequent as that of the left lung. In 35 cases the right, in 18 cases the left lung was affected. The localization of the consolidation is given in the following table:

Lesion	Right	Left	Total
Apex	5	3	8
Upper lobe	4	5	9
Middle lobe	9	..	9
Lower lobe	13	7	20
More than one lobe	7	6	13

It has been shown that lesions which are apparently healed or fibrous can occur in very young children, and among our examinations two children were found who, at the age of two years, showed marked circumscribed subclavicular retraction on the right side, over which the percussion-note was distinctly short, with a harsh character of the inspiration. Both children were exposed to infection in the family, and reacted locally to the vaccine; but stethoscopic examination failed to detect any focal reaction in the lung-areas mentioned. One was reexamined after three, and the other after six months without any change being discoverable in these physical signs; they were accordingly classed as "stationary."

In this connection the records of four other children appear to us to be of interest, their ages being four, six, seven and nine years respectively. In these children the lungs were found free from physical signs on first examination. All of them reacted positively to the vaccine, the reactions being attended by slight fever in two, and all had shown remarkable general improvement when they were reexamined three, six, forty-two and forty-two months later, respectively. On reexamining these four children, physical signs were found, however, in the form of marked circumscribed retraction over which the percussion-note was now short and the vesicular quality of the respiration was

[21] L. E. LaFetra; Trans. Washington Tuberculosis Congress. 1908, II, 361.

replaced by a more or less harsh character. These signs had not been found on the occasion of the first examination, and no examination had been made for the purpose of ascertaining the occurrence of focal reactions after the children had received their doses of the vaccine.

That these retractions correspond to what we believe to be and have recorded as healed lesions, we have reason to infer from other cases in which we found physical signs before vaccination, which thereafter disappeared entirely, while at a later examination we noted the same changes. In illustration of this, we may refer to a case examined June 25, 1911, before vaccination, the record showing "marked physical signs in both lungs to 2nd rib anteriorly and spine of scapula posteriorly"; the details were shortness of percussion-note, more marked on the right side, with rough inspiration; prolonged expiration, especially on right side posteriorly. On reexamination, March 2, 1912, the record reads "no physical signs, except that the percussion-note is still slightly short over the right apex posteriorly." Reexamination, April 25, 1914, gave the same record with the addition "slight subclavicular retraction, right side." Reexamination, April 18, 1915, same record with "retraction right side anteriorly *marked*, and slight retraction also on left side. Percussion-note on right side posteriorly, normal; over retracted areas short; respiratory sounds normal."

Another case of interest, on account of the very rapid disappearance of physical signs after the intravenous administration of the vaccine, is that of Miss W. O. J., age eighteen years, with physical signs marked on the right, and less marked on the opposite side. Temperature 99.4° F., pulse 96, no enlarged glands. Two small doses of vaccine (1 mgr. with temperature rise to 100° F., and eight days later, 2 mgr. without rise in temperature) were administered intravenously, after the first of which the patient had a marked focal reaction. On reexamination, seven days after the second dose, absolutely nothing was found in the lungs; the patient had gained five pounds in weight during the fifteen days since receiving the first dose, and since then her temperature has remained normal.

The interest in two other cases lies in the satisfactory results from much smaller doses than those which we have given ordinarily as single full doses:

Mrs. N. M., age about thirty years; referred to us on account of pulmonary tuberculosis, diagnosed by her home physician. Our examination showed physical signs marked in one lung, and slight in the other. The patient had never been strong and had, more recently, shown loss of appetite and of weight; her temperature was normal, she had no cough, no expectoration. She received 3 mgr. of vaccine, which was followed by local, general and focal reactions, after the subsidence of which we acceded to her request that she be permitted to return home temporarily, in order to arrange for a more prolonged stay and treatment in the institution. Her return for this purpose was deferred on account of the continued general improvement which she experienced. This patient came back, however, about one year later for reexamination, and then no necessity for further treatment could be discovered, the physical signs which had been recorded previously having disappeared. Nothing abnormal was found except a circumscribed retraction on the right side subclavicularly, over which the percussion-note was short and the respiration faintly bronchial. She had gained twelve pounds, and stated that her health had never been so good in the past.

V. B., girl, age ten years. Both lungs showed considerable involvement, more in the left than in the right. The left lung presented changes on percussion and auscultation, anteriorly to the third rib, posteriorly to the lower angle of the scapula, accompanied by moist and dry râles and rhonchi.

This child was brought to us by a neighbor who knew little of her previous history, except that her mother had died of consumption. The girl herself apparently did not have sufficient interest or intelligence to answer related questions correctly. She seemed listless, was badly nourished, pale and sallow; her chest showed marked deformity and there was a well-defined spinal curvature. Temperature 100° F.; pulse 96; respiration 24; weight 47 lbs.; both cervical and the left axillary groups of lymph-glands were enlarged; she had cough, and appeared to swallow her expectoration.

To Dr. E. R. Stitt of the United States Navy Department, who examined the girl with us, her appearance suggested a complicating hookworm affection, and he expressed himself pessimistically as to the probable improvement of her lung affec-

tion, from treatment with the vaccine. The child, however, received 1 mgr. of vaccine subcutaneously and, on her return on the following day, a well-marked local and general reaction was found; we also discovered a focal reaction in the right upper lobe, the râles and rhonchi preventing a definite conclusion in this respect in the left lung. We then obtained a specimen of sputum, which contained tubercle bacilli.

It was our intention to treat this girl by giving additional doses of vaccine; but she returned only once thereafter for a comparative serum test. Our records show the following:

May 1, 1913; before vaccination:

Agglutinins 1:10; Precipitins 1:200; Specific Amboceptor negative; Bacteriolysis negative; Blood-alkalinity 0.85.

May 6, 1913; five days after receiving 1 mgr. of vaccine:

Agglutinins 1:60; Precipitins 1:100; Specific Amboceptor 1:4 plus; Bacteriolysis 1 plus; Blood-alkalinity 1.0.

Later attempts to find the child were unsuccessful; the family had moved, and she was lost from observation for the time being.

In *November, 1914,* we succeeded in locating her and had then an opportunity for a reexamination, which showed a remarkable degree of improvement in all respects, which seemed to have been continuous. Her appearance was good, the pale, sallow color had disappeared; temperature was normal; pulse 88; respiration 18; weight 52 pounds. She was bright and lively, had lost her cough and expectoration. The examination of the lungs showed slight percussion-changes limited to the apices; subclavicular retractions on both sides, over which the vesicular respiration was weaker, with a soft bronchial character on expiration. There were neither râles nor rhonchi in either lung, which appeared normal except as stated. Two reexaminations made since that time resulted in the same findings; the child's weight has increased further and was 60 pounds in May, 1915, that is, two years after our first record was made.

Many more cases could be added which would prove of interest; in fact, there are few, if any, which lack in this respect when the results are considered which followed upon the administration of one or a few doses of vaccine. They appear to

us ample to justify our expenditure of time and effort, even though a comparatively large amount was necessary. In our opinion, these results should arouse and engage the interest of all students of the tuberculosis-problem, with a view of studying and of applying the method which has yielded them.

We have certainly shown that the early manifestations of tuberculosis-infection are frequent enough to justify a thorough search for cases similar to those that we have vaccinated; and they can be found in great numbers in tuberculous families. We believe that the physician who will search them out will find not only no opposition but, as was our experience, he will meet with cordial cooperation on the part of the parents and interested friends. No one would doubt that, if tuberculosis has actually been acquired, it is best to ascertain the fact positively as early as possible, if for no other reason than for the purpose of instituting a routine of general hygiene, in order to afford nature the best opportunity for overcoming the disease. This she does in a large number of infected children, and often without appreciation on the part of anyone, because the existence of the infection or disease is not known or is but barely suspected.

We have again shown as others have done before us that, with a case of open tuberculosis in the family, young children living in the same home rarely, if ever, escape an infection, and there is ample evidence that, even without such constant and continued exposure, most children have acquired an infection by the time they approach maturity. This being now conceded on every side and the present tuberculosis-mortality averaging only about fifteen per cent of all living, it is plain enough that the great majority of people escape the development of clinically manifest disease. It is then the minority, of about fifteen per cent, which we need to safeguard against the event of infection and disease, but although it is a minority, it still amounts to millions of persons even in our own country alone. We are thus thoroughly aware that the majority of the children whom we vaccinated might have remained free from actual disease, and we do not fail to appreciate that, as yet, there is no conclusive evidence, nor can there be any at this early date, that our intervention will surely protect our vaccinated children during the entire remainder of their lives.

The service which we have rendered consists, therefore, at least in an improvement of the physical condition in instances in which this had been below the normal standard. Having observed no serious relapses from the attained improved state of health in those whom we have had an opportunity of re-examining, we feel that, even if this were all that we accomplished, it would certainly have been worth the while.

Since these benefits have accrued from so simple a procedure as the administration of one or a few doses of vaccine, which at the same time gave us the certain diagnostic information for which, in the very early stages, we must otherwise have recourse to the tuberculin test, we feel it our duty to continue our work and to urge the practical cooperation of the medical profession, since the clinical evidence, as far as it is available at the present time, is sufficient to support our contention. This evidence must, in the end, form the crucial test and criterion of the merits of our method, when it is applied to tuberculous children.

When we commenced our studies, no such clinical evidence was at hand. WE BELIEVED THAT WE KNEW that our Watery Extract of Tubercle Bacilli influenced the clinical course of tuberculosis directly and favorably, and that a like influence was obtainable from other similar preparations; but under our mode of its therapeutic administration then, this was prolonged over periods of months and even longer. In our opinion, it would be futile to recommend a like course of prolonged prophylactic treatment for children who are not yet manifestly in need of it, because the advice to have it administered would usually be neither acceptable nor followed.[32] What was needed was a rapid, simple and effective method, and we are convinced that we have found it, although by a very circuitous route which practically led us back to where we started in 1896, since ultimately we discovered that the Watery Extract of Tubercle Bacilli, introduced by us in 1897, will do all that we can do

[32] Petruschky's success in inducing those interested to submit to a long and protracted course of prophylactic treatment, is no argument against our position, because his results cannot be duplicated easily in the general population. Both he and Kutschera worked in isolated communities or institutions, and had therefore less difficulty in continuing their tedious course of treatment for the necessary length of time than they would find in cities or in country districts among people at large.

with our present vaccine, provided it is given in sufficiently large doses. We had even then built better than we knew, and it would then have required only a suitable adjustment of the several constituents of the Watery Extract to perfect the preparation to the present standard of the Vaccine; but, above all, it would have required the courage to administer the Watery Extract in adequate doses. The lack of this courage, we believe, even now handicaps us somewhat, because we are still careful to reserve a full, single dose for a selected class of individuals, in order to safeguard others as far as may be from severe and possibly harmful general reactions. This lack of courage was, and is, however, not peculiar or limited to ourselves; it was then, and is still, shared by the great majority of those physicians who are interested in the specific treatment of tuberculosis, even though we were told early and repeatedly by Professor Koch that the best results followed the administration of tuberculin, when the doses were large enough to cause decided reactions. One of us discussed this very feature with Koch personally at the time of his visit to this country, in 1908, and he still insisted upon the correctness of his position.

Our experimental studies with the Watery Extract, to which we referred on p. 123, also misled us by our failure to obtain sufficiently uniform results in treating and protecting animals. At that time, however, we had no guide whatever for regulating the administration of our remedy and, on going over these experiments, in the light of our present experience, we can only appreciate that the results then could not have been other than we found them, and that they would be the same again with our present vaccine under a like mode of procedure, because the animals were, as a rule, treated improperly, either with doses that were unduly small, or administered too frequently, or continued for insufficient periods of time; or then because they differed individually in responding to the treatment. Aside from these causes for failure, it is not possible, at the present time, to estimate to what extent our results were obscured by pseudo-tuberculosis which has hampered us so greatly during the last two years; and at that time it would have been more difficult to recognize the existence of one or two types of this affection; in fact, its presence was not considered at all.

CHAPTER VIII

THE SPECIFIC TREATMENT OF PULMONARY TUBERCULOSIS

The specific treatment of tuberculosis has the object of producing a bacteriolytic immunity against the tubercle bacillus, or of increasing it where it exists, and also of stimulating the circulation in the tuberculous lesions. These lesions differ not only in size, but also in their character which may vary from the earliest degenerative changes in the protoplasm of the tissue-cells to complete necrosis. In the open stage of tuberculosis the destructive lesions are subject also to infection by other pathogenic bacteria; various types of inflammation are apt to occur, locally and in more distant parts, through the aspiration of secretions; and finally other complications often are present in non-tuberculous organs.

Under these circumstances it cannot be expected that the action of specific remedies will be equally favorable in all cases; on the contrary, the character of the lesions and the nature of existing complications must necessarily govern the clinical results, and it is evident that their nature and extent may preclude any benefit from specific treatment, or that the removal of complications may be required before it is possible to realize a favorable influence.

It is therefore necessary to select the patients, for whom specific treatment is taken into consideration, with reference to the pathological changes which are found, and to the action of the remedy which is to be employed. We are fully cognizant of the fact that the extent and nature of disease-processes, especially of internal organs, as a rule cannot be determined with absolute certainty, and we are in sympathy with the humane attitude of giving all patients every possible chance, even if we may not be able to appraise it fully with the evidence before us; yet we believe that it is possible to recognize cases in which the uselessness of specific treatment is unquestionable from

the start. We hope that the detailed considerations which follow will aid some of our readers to a better understanding of the subject of specific therapy in tuberculosis, more particularly with reference to its possibilities and its limitations.

THE SELECTION OF CASES

The indiscriminate use of tuberculin, after its introduction in 1890, on all tuberculous patients, especially those who were in the advanced stage of phthisis, formed the chief reason for its early abandonment, because of the many failures and untoward results to which it led. The disappointment, which was experienced at that time, would probably not have been suffered if the employment of the remedy had been limited to those cases which Professor Koch had pointed out as suitable. With a better insight into the laws of immunity, and with increasing clinical experience in tuberculin therapy, its diagnostic and therapeutic value is now understood better.

We have described the principles which govern us in the selection of those early-stage cases which we consider suitable for vaccination, on p. 142, and have shown that it is possible to avoid any danger that might arise from the administration of comparatively large doses by following a proper mode of procedure. We are confident that the following further discussion of the subject will enable the general practitioner to adopt a safe course in the specific treatment of existing active and progressive tuberculous disease, where this treatment is indicated, and to realize that the administration of the toxic products of tubercle bacilli is at least useless in some forms and phases, also that it is contra-indicated in certain cases, because larger doses may be productive of harm, while small ones cannot confer any benefit.

We have tried to make it clear that vaccination with a single dose should be limited to cases in which the evolution of an existing infection either appears to have been checked at an early period or has not yet progressed sufficiently to cause marked evidence of disease. In cases in which the disease has advanced further and in which prolonged treatment is required, the selection of the patients for specific treatment is not quite

so simple, be it with our preparations or with others, and clinical observers have arrived at widely differing conclusions in this respect. It is possible that some of the good results in more or less advanced, febrile cases, which have been attributed, in part or entirely, to specific treatment, may have coincided with a natural amelioration, the cause of which had been overlooked; this may be the case, for instance, if a caseous abscess breaks into a bronchus, or if the absorption-fever diminishes or disappears when better drainage is established from secreting pulmonary cavities, or again if inflammatory complications subside. However this may be, in common with other clinicians, we have made unusual observations in respect to striking clinical improvement under the administration of specific remedies, which could not be reproduced uniformly in other patients, although we could discover no good reason to explain our exceptional successes or our unforeseen failures.

In a paper read recently by one of us before the Ohio Valley Medical Association, the writer took occasion to discuss a case of this kind, which had been reported to us by another physician, with permission to use it for publication. Although the patient in question had presented signs and symptoms which are characteristic of the advanced, hectic type of phthisis, he had made a clinical recovery under specific treatment in the course of four months. Several months later death occurred from injuries received in an accident, and the autopsy confirmed the clinical result in all respects. In this case there was, however, evidence that the acute symptoms and signs were probably due to a pneumonic complication, that the tuberculous disease was not as extensive as had been believed, and that it was limited chiefly to the right upper lobe, which was the seat of a cavity that supplied the tubercle bacilli found in the sputum of the patient, together with many pneumococci. Assuming that this view corresponds with the facts, there is not anything mysterious or even very remarkable in the clinical recovery from phthisis, or in the spontaneous recovery from the pneumonic complication which had caused the active symptoms, both of which were shown on autopsy.

This case may be considered as having the value of an experiment upon the human subject and is of importance since it is never possible during the life of a patient to determine

the existing pathological changes with absolute certainty. The best that can be done, therefore, is to bear in mind any unusual results or observations until an explanation is afforded by further experiences, either personal or on the part of other observers.

While the course of tuberculosis under any method of treatment can never be foretold with certainty, except perhaps in those very early cases that are suitable for vaccination with one or a few doses, it is possible, by proper examination and study, at least to exclude certain cases which are clearly unsuitable for specific treatment, and to select others in which a trial is justifiable or will become so after the removal of existing complications; whereas, in still others a reasonable assurance of benefit is afforded and important aid may be expected from this treatment in securing the arrestment and, ultimately, the clinical cure of the disease.

In attempting to aid other physicians in the proper selection of clinical cases that may be found eligible for specific treatment, we must of necessity be governed by our personal experiences. What we shall say applies, however, not only to our vaccine or the Watery Extract of Tubercle Bacilli, but also to all other preparations of this class, whether these be the old tuberculin of Koch or another tuberculin, or an emulsion of tubercle bacilli.

In the light of our personal experiences, specific treatment is to be withheld in acute generalized miliary tuberculosis, in all acute or rapidly progressive cases of phthisis, and during acute complications whether these be of tuberculous or of other origin. If acute processes of any sort intervene in cases under specific treatment, this should be suspended until after they have subsided. We exclude therefore from specific treatment, for the time being, all febrile cases of phthisis and of other forms of tuberculosis in which we believe that products from breaking-down lesions are being absorbed into the circulation in large amounts. Our reason for this is based upon the fact that, on making critical examinations in such cases, we have usually been able to find tubercle bacilli in the circulating blood, or we succeeded in demonstrating specific toxins in the blood-serum by testing it against an immune serum the antibody-titer of which had been determined previously. Apart from these laboratory

demonstrations, these cases can be recognized clinically by physical examination and by an analysis of the coexisting symptoms, especially the fever. It is not necessarily a condition that a patient should have been ill for a long time, or that he be greatly exhausted or emaciated; the active process may be quite recent and it may subside again. For the time being the patient is certainly not in need of more specific toxins in the form of a tuberculin or of a specific vaccine. Our therapeutic problem is rather to limit their further access into the circulation, if it cannot be arrested entirely. We therefore place the patient in a condition of absolute rest; we try by dietetic and hygienic measures to conserve his nutrition and strength as far as possible, and to overcome troublesome symptoms with other appropriate remedies.

If the area which is undergoing destructive changes shows no signs of delimitation for several weeks after such symptomatic management, if the destructive process appears to be confined to a portion of one lung, and if there is no decided improvement in symptoms, it will be proper, in the absence of contraindications, to consider an artificial pneumothorax, with the prospect of limiting the absorption or of arresting it entirely.

Acute destructive processes sometimes occur in multiple foci and may then belong to the terminal stage of caseous pneumonia, or they may coexist with older cavernous and fibrocaseous processes which are located in the same, and oftener in the opposite lung.

In patients who present less acute symptoms, but who still have daily rises of temperature, exceeding 100.5° F., it may be more difficult to find the destructive lesion, especially if the physical examination and the study of the case are not exhaustive. A caseous focus may be in the course of softening and, for the time being, no sign of localized râles or of moisture may be audible during ordinary respiration, the signs that are found belonging either to the closed early stage or to older changes, with or without evidence of a cavity. If the examiner has made it an invariable rule, as we have done many years ago, never to consider a case to have been investigated sufficiently until a reasonable explanation has been found for all objective findings and for all subjective symptoms, he will reexamine the chest more critically if possible, in order to ascer-

tain whether caseous softening is actually and alone responsible for the fever. He may then discover an area over which, during deep and rapid inspiration, or after coughing, auscultation betrays the impending excavation by at least a few moist crepitations or by a mucous click, strictly limited to a certain area, and as a rule situated in an upper lobe; even if this is not the case, it will be advisable to wait and try again, and in the meanwhile to make a search in other directions. At all events, in our judgment it is best to withhold specific treatment, to make use of the benefits of more or less complete rest, and to treat the non-tuberculous complications if any are found.

In instances like those here considered, the actual conditions may of course remain in doubt. If the signs are slight and not strictly circumscribed, they are not necessarily due to caseous softening, and the cause of the fever may remain obscure, at least for a time. Whether or not it is advisable to make a trial of specific treatment in such a patient, depends upon other associated physical signs, and upon the character of the associated symptoms.

Summing up the contra-indications for the administration of specific remedies in tuberculosis, we may lay it down as a general rule that we exclude all patients who have daily temperature-rises to 100.5° F. or over, until we are reasonably assured that the fever does not depend, at least in part, upon the absorption of products of disintegration from breaking-down caseous foci. We also bar patients who have non-tuberculous febrile complications, including the so-called mixed infections which may be deemed responsible for the existing fever, until we have reduced it by appropriate treatment, including the administration of autogenous vaccines in cases of mixed infection. We further exclude all patients the results of whose examinations indicate no reasonable prospect that material improvement would follow, on account of the extent and nature of the destructive processes, of irremovable complications, or of general emaciation and exhaustion.

The relation of the fever to a progressive eruption of new tubercles has to be considered if a lack of normal vesicular respiration is found over areas that are peripheral to older lesions, or in the opposite lung, and if the inspiratory sounds are decidedly rough, coarse vesicular, without distinct râles,

or perhaps giving only a suggestion of moisture by conveying a sense of stickiness. Very fine crepitation may be appreciable toward the end of inspiration or on coughing. The temperature may reach 100.5° F. or over if a larger area is the seat of forming tubercle. In such a case active immunization will be of benefit.

Having eliminated other causes, including peri- and endocarditis, subacute or chronic colitis, fecal impaction, peritonitis, etc., in a patient whose physical examination does not warrant the view that caseous softening is in progress, who is not yet seriously emaciated or exhausted, and in whose case improvement might reasonably be looked for, were it not for the fever, we feel justified in resorting to specific treatment. In this event it will, however, not do to depend upon extremely minute doses, but it will be preferable to start, as in other less obscure cases, with at least one-half or even with the full usual initial dose and follow the ordinary mode of increase, controlling each dose by comparative observations for focal reactions in the suspected and in other lung-areas, while attention is paid to the other clinical symptoms at the same time. Under such a mode of procedure we are not likely to do harm, and numerous instances could be cited from our records in which the fever subsided, sometimes abruptly, in connection with a marked general and focal reaction, or more slowly without it as the treatment was continued.

Other doubtful cases present themselves in which trials are justifiable, and mention may be made of chronic cases of phthisis without marked fever or without any fever, but often characterized by insufficient nutrition and strength, although the patient may take a sufficient amount of food. When the lack of nutrition and assimilation is not accounted for in these cases by existing digestive complications, or by a greatly reduced respiratory capacity, which may result from extensive bilateral disease through fibrosis, pleural adhesions, etc., a trial of specific treatment is permissible on the supposition that the loss of weight and strength is referable to a specific toxemia; in these cases the expectation of improvement is often justified by the events. In still other more or less advanced cases the fever may be due to an associated infection without any material change or increase in physical signs, which perhaps indicate chronic

interstitial pneumonic changes. If we succeed in recognizing the related bacteria or in including them in the culture which is made from the sputum of the patient, it will yield to an autogenous vaccine, and then the specific treatment of the tuberculous processes may be attempted with better prospects of success.

In regard to the question in what class of cases specific treatment is properly added to our other methods, it is possible to be more concise and to state that all cases are suitable for this form of treatment that have not reached the open stage and in which this stage is not impending; also all cases that have open lesions which are not progressive or rapidly extending, which are draining or have ceased to secrete and in which acute or subacute inflammatory complications are absent. In all these cases fever is intermittent in type, if it is present at all, and does not usually exceed 100.5° Fahrenheit.

By open lesions we understand tuberculous excavations, abscesses, ulcerations, which communicate directly or indirectly with the exterior. We therefore assume an open tuberculous lesion to exist, even though it cannot be demonstrated or located, if tubercle bacilli are present in the secretions and excretions, or if they appear in the blood. We infer the existence of an impending open lesion upon the same or on similar evidence as that which indicates a closed or forming abscess or one in which secretions are retained, regardless of its nature and origin, for the diagnosis of which in internal organs we are guided by the results of physical examination and by reference to existing symptoms. We consider an open lesion to be non-progressive, or not rapidly progressive, when the signs of cavity, abscess, or ulceration show no evidence of an increase in size on repeated examinations made several weeks apart, and we assume that adequate drainage has been established if no fever is present or if it is slight and does not show frequent or marked increases or other signs of retained secretions.

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A review of the correlated physical signs and symptoms, which require attention in the selection of cases for specific treatment, may elucidate our position further in regard to the therapeutic administration of products of the tubercle bacillus.

Destructive changes in tuberculous tissue are necessarily

preceded by necrosis and caseation, which is the most common destiny of tubercle, unless the infection is resisted specifically or lacks in virulence. If the virulence is primarily slight, or if it is reduced by the activation of a specific resistance, the reaction of the tissue-cells to the tubercle bacillus and to its secretions may prevent caseation or retard it; in the former case no clinical symptoms of disease appear, and in the latter event the tissue-reaction leads to more or less organization and encapsulation, in consequence of which the symptoms are correspondingly slight and obscure. After the existing symptoms have attracted sufficient attention to induce the patient to seek medical aid, small caseous foci or larger caseous areas may be supposed to exist in most cases of tuberculous disease. These caseous foci often remain stationary for long periods, undergoing no changes that can be discovered clinically; but they are apt to increase in number or to coalesce with adjacent tubercles and other caseous foci, and it is in this manner that the larger fibrocaseous nodules and masses are formed which are characteristic of chronic tuberculosis of the lung, whereas, without caseation or recent tubercles, the final anatomical changes are those of fibrous thickening and of shrinking, and constitute spontaneous healing. Patients whose lungs present such purely fibrous lesions are not apt to have symptoms which cause them to consult a physician, and are examined only by accident, perhaps after having sought the advice of a physician for an unrelated ailment, during examination for life insurance, or on some similar accidental occasion. On physical examination one finds a decidedly dull or practically flat percussion-note, and bronchial respiration that is free from râles. If the area is large enough and does not contain normal or emphysematous lobules, the respiration lacks all vesicular quality; marked retractions are noted, which correspond with the location of these physical signs.

Fibrocaseous foci and infiltrations, in which the percussion-note is relatively dull, cause no such marked retractions; they are often surrounded by or have scattered through them more or less air-containing tissue which may be the seat of more recent tubercles. The respiration is usually of a broncho-vesicular type; often the inspiration is sharp rather than harsh or bronchial; and some catarrhal signs may be present; the temperature

is usually normal, or it is elevated slightly, rarely over one degree Fahrenheit, unless destructive lesions are present elsewhere or the fibrocaseous lesions are beginning to break down.

The signs and symptoms of caseous softening are not always sufficiently distinctive in their onset to make it possible to differentiate and recognize them easily, and it is often only by weighing all obtainable evidence or by repeated reexamination that an approximately correct conclusion can be arrived at.

In cases that are apparently still in the so-called closed stage, the first evidence which should arouse suspicion of the onset of destructive changes is the appearance or the increase of fever. The presence of fever does not indicate of itself whether the absorbed product comes from a caseous focus which is softening, or from a locality where an eruption of new tubercles is taking place. It may also be due to the activity of other bacteria which give rise to non-tuberculous complications within or outside the organ which is believed to be the seat of tuberculous disease. Although the type of the fever affords a clue, other symptoms and signs must be taken into account as well. The first step naturally would be an effort to exclude other than tuberculous changes: for instance, acute or catarrhal affections of the air-passages or gastro-intestinal disturbances which have developed or increased. Our inquiry is the more likely to elicit the related cause promptly if the patient is already under our care and if we have complete records; in the case of a first consultation, we must depend in part upon the information which the patient himself can supply, and this may be misleading. After the consideration of all possible non-tuberculous causes, the tuberculous factors in the production of fever will require investigation. The early physical signs and the symptoms which they produce have been discussed in the consideration of the selection of cases for vaccination. If in more advanced cases, the examination of both lungs shows the abnormal area or areas to be circumscribed, if they are free from râles, rhonchi or lesser signs of moisture, if these signs cannot be obtained on deep inspiration or on coughing, the conclusion is justified that no evidence exists of caseous softening or of impending excavation.

When the temperature is intermittent in type and does not materially exceed 100.5° F., the possibility of progressive erup-

tion of tubercles is next to be thought of. Such an eruption may account for the fever if, adjacent to apparently older lesions, over which the physical signs are marked, more recent areas are found over which a practically normal or but slightly short percussion-note is elicited, while, on auscultation, only rough, coarse vesicular inspiration is noted, which has no bronchial element and is free from râles or is characterized only by giving an impression of stickiness. An extension of tubercle-formation becomes more probable, if the transition is not well defined from an area of marked physical signs to one where the respiration is of normal soft vesicular quality, if the signs diminish gradually and if, on receding from the supposed older lesion, the percussion-note becomes normal, while abnormal auscultatory signs are still manifest. If no recent tubercle-formation can be assumed, it is probable that caseous tubercles or fibrocaseous foci are responsible for the fever, and that specific bacillary products are being absorbed from them.

It is to be remarked further that caseous softening may be in progress in the older lesions, and that new tubercles may be forming in the periphery. This is not at all uncommon; it is the borderland that lies between the area of well-marked physical signs and the normal lung in which the tubercle-formation is likely to be comparatively recent.

Occasionally, however, we are surprised by finding the cause of the fever in distant tuberculous or non-tuberculous complications, where they have least been expected; we remember numerous instances of this kind in which the existence of genital and other pelvic diseases, perirectal abscess, fecal impaction, chronic appendicitis, etc., accounted for it. If, even after eliminating such distant causes and after going over the entire ground repeatedly, the origin of the fever still remains obscure, it is proper to make a trial with specific treatment. Sometimes it happens that the fever subsides while we remain uncertain, or that the appearance of other symptoms points the way. In other instances the apparently negative results of our previous examinations become less so, and eventually they become decidedly positive. If softening is actually in progress, its location betrays itself in most instances by the advent of more definite physical signs, chiefly râles and rhonchi of smaller size. These catarrhal signs extend to the adjacent tissue, and then the fever

is apt to increase; it does not subside even if the patient is kept at rest in bed, or it does so only in a slight degree.

If the catarrhal manifestations are looked upon as an evidence of a continued focal reaction in response to the local effect of bacterial toxins which are diffused from a softened center, their disappearance may be interpreted to mean that the softened focus has discharged outwardly; or, if it is very small, that it has become exhausted by absorption or has become encapsulated more firmly. On the other hand, an extension of the catarrhal signs is not in itself to be taken as signifying that the lung-area which is breaking down is becoming larger. It may even happen that catarrhal signs appear in the opposite lung, if this is also the seat of tubercles, because these lesions may take part in the spontaneous focal reaction to bacterial products which have been absorbed and have reached the general circulation. We mention this for the information of those physicians who have not often been in a position to watch these local manifestations and to observe their progress, and who are therefore likely to apprehend that extensive excavation is taking place on both sides, until they are relieved of their fear after the pent-up material has found an outlet and free drainage is established. The distant catarrhal signs then disappear, and ultimately only one small cavity may be discoverable, which may be so small that cavity-signs are not very definite or typical, and never become so.

We recollect cases, however, in which no change took place in the existing physical signs, and one particular case in which the period of uncertainty continued for over one month, without cough or expectoration. In this patient the fever-period terminated with a copious hemoptysis and with the establishment of free expectoration in which tubercle bacilli were found in great abundance without the association of other bacteria. A cavity could be demonstrated at the site of the suspected lesion on examination about one week after its perforation into a bronchus. In this case the temperature had gradually increased to 102° F.; for several days the onset of the fever had been accompanied by a moderate chill, the subsidence of which was followed by a night-sweat. All these symptoms disappeared with the evacuation of the cavity.

It has been mentioned that the type and the course of the

fever may aid in arriving at a conclusion in regard to the nature of the local changes in the tuberculous lesions. As a rule, the fever is intermittent, the rises take place slowly, often late in the day, and an approximately normal temperature is reached in the evening. While this is the case also in slighter febrile manifestations which have no relation to caseation, in the conditions under discussion the fever is apt to be higher and to exceed 100° Fahrenheit. If it can claim any characteristic features at all, these may be found in its persistency and in its tendency to increase slowly and to appear earlier in the day; also in the fact that it does not yield to rest, even if this is absolute, that its onset often is accompanied by a slight or more decided sense of chilliness, and that the temperature is frequently subnormal in the morning.

In the closed stage cough is not a prominent feature unless active symptoms of caseous softening have been initiated by acute inflammations like influenza, pneumonia or bronchitis, which frequently act as exciting causes. In such cases the cough, which attends the acute affection, usually improves after a time, as do also the more diffused catarrhal signs for which the incidental pulmonary affection was responsible. The physical signs, which were present at first perhaps in both lungs, disappear gradually; they become limited to the tuberculous changes and are most marked in the area which is the seat of the softening and where the catarrhal signs persist. During this time the sputum is free from tubercle bacilli, but it shows other microorganisms, some of which are likely to stand in relation to the acute complication. If cough persists, it is usually slight, perhaps only a hacking, but reflexes from the involved bronchi or from pressure may cause more or less severe paroxysms of coughing in exceptional cases.

Certain symptoms of pleural irritation may also be of aid in reaching a correct conclusion as to the presence or absence of caseous softening. Although they are the same, or are similar to those which are observed in afebrile patients in connection with healing and fibrosis especially in the apices, in the latter case they are usually associated with marked supraclavicular retractions corresponding to the seat of a dry or shrinking cavity or of a more diffuse fibrous area, whereas such retractions are not apt to be present over the seat of an impending excavation,

at least not in so marked a degree. The patients speak of a sense of pressure, of dull aching or discomfort which corresponds more or less definitely to the lung-area under suspicion, or which is referred to the corresponding shoulder. In the case of impending excavation, however, when the pleura is not yet bound down firmly by organized adhesions, more marked signs of dry pleurisy may be manifested by crepitation, the pleural origin of which is suggested by their evident nearness to the surface, and by the observation that they are apt to disappear after a few rapid inspirations. Acute stitch-like pain or distinct friction may at times be elicited on deep inspiration and are indicative of subpleural changes in the lung. Instances are not infrequent in which pleural pain and signs occur in connection with caseous softening and are the first to attract attention. They are not necessarily confined to the locality where tissue-destruction is in progress and need not correspond with it, but may be located in the base of the pleural sac when the related changes are higher up or in the apex, and they may be attended by exudation. An acute pleurisy, with or without exudation, often originates in this manner without apparent cause, and the latter is likely to remain unrecognized in cases in which there had been no opportunity for making a careful examination of the upper part of the chest before the exudate has reached an amount which interferes with it.

It is necessary to bear in mind that, in this stage, the fever must differ in degree, accordingly as softening occurs in one or in multiple foci, according to the extent of the involved area, and to the state of the peripheral tissue as it permits or retards absorption. The physician must be prepared to meet cases in which little or no absorption takes place, on account of fibrous changes or because the afferent lymph-paths are blocked, and in this event the temperature is influenced but little or not at all. He can then better appreciate a clinical history which fails to make reference to previous symptoms that may account for the presence of an old secreting or even dry cavity which he finds on a first physical examination, the patient having accepted the attending cough and expectoration as being due to cold. On the other hand, existing symptoms may increase rapidly; the temperature may assume a severe hectic type and the patient die of exhaustion, especially when many small foci

break down and open into small bronchi that are easily obstructed and afford little or no drainage. Dry or exudative pleurisy occurs frequently in such cases, and we have even seen pneumothorax following the perforation of the pleura from a small necrotic focus underneath. If cases of this kind come to autopsy, larger lung-areas or entire lobes are found riddled with small caseous foci in various stages of softening; some of them barely visible; larger ones, the size of a pea or bean or exceeding it, interspersed in the parenchyma which is in a state of reactive inflammation, while the bronchioles and the smaller bronchi are blocked with caseous detritus and mucus.

Not all destructive processes in the tuberculous lung take place in such a manner that the caseous softening at first gives rise to a larger or smaller tuberculous abscess which then perforates into a bronchus of larger caliber and leaves a pulmonary cavity, or which, without discharging itself, becomes walled off further by a fibrous capsule within which the softened detritus becomes inspissated, with or without the deposition of calcareous or cretaceous material.

Tuberculous ulcerations may occur primarily in the mucosa of larger or smaller bronchi. It is particularly at the junction of the terminal bronchi with the alveoli that tubercles may form, caseate and break down very early and result in minute lesions which are in communication with the bronchial system almost from the beginning. From the ends of terminal bronchi, the infundibula and alveolar spaces become speedily implicated, and the formation of tubercles and caseation may progress by continuity in an outward direction to the small bronchi from which they branch off. Thus one or several lobules may be destroyed; as their contents pass outward, the infection extends to adjacent lobules, the secreting ulcer becomes larger and constitutes a small cavity. Unless these changes are near the surface of the lung, physical signs may not be present before the appearance of slight cough, or even before the occurrence of a so-called initial hemoptysis, or before tubercle bacilli have been demonstrable in the scant secretions from larger bronchi into which the discharges from the minute foci have gained entrance. If the destructive processes originate in this manner, fever may be slight or absent, or it supervenes temporarily when the small bronchial communication is obstructed by swelling or by secre-

tion; but, inasmuch as cough and bacillary expectoration occur very early, these cases are not so perplexing to the examiner, as concerns diagnosis, although he may not discover and locate the existing lesion immediately by the physical signs; they respond, however, to specific treatment in a very satisfactory manner.

In the examination of phthisical patients, the diagnosis itself does not often present serious difficulties even to those who have little practical experience, unless it is in instances in which the active disease is arrested to that degree that cough and expectoration are but slight or have disappeared, at least for the time being. When no sputum is available or when it is only catarrhal and free from tubercle bacilli, the result of the examination of the lung may leave a doubt concerning the origin of the physical signs which persist; but even when their tuberculous origin is unmistakable, the question may arise whether additional treatment or other precautionary measures are required. In all these cases our remarks on the diagnosis of the closed stage apply; in others, the examination is made not so much for the purpose of actual diagnosis, as rather for that of prognosis, with reference to the therapeutic resources which are at our command.

In ordinary cases of phthisis, the examination has the object of taking an inventory of the patient's disabilities, as they are represented by the tuberculous processes and by the complications that are found in the lungs and in other organs, and of their nature, extent and apparent activity, on the one hand; and, on the other hand, of the natural resources which he may still possess and which may be made fully effective under our guidance and with such aid as we may be able to give by more direct therapeutic measures. Such an inventory is afforded by the recorded results of our examination. The more comprehensive and searching our inquiry has been, and the more experience we have had in checking up the supposed balances by previous observations in other cases, the more apt are we in individual cases to arrive at a correct estimate of the future course of the disease.

It is therefore necessary to make the inventory as complete as possible, so that nothing may be omitted which can influence the balance, and this demands not only the ability to recognize

the physical signs and symptoms of tuberculosis, but also the faculty to interpret them and to appraise their direct and indirect influence upon the course of the local disease, as well as upon other organs and other functions, in the light of the teachings of pathological physiology.

Coexisting non-tuberculous complications likewise require painstaking analysis, especially in relation to their origin, since they may prove not to represent independent affections but rather secondary effects of the tuberculous disease itself. On the other hand, an independent disease may exert its effect directly or indirectly upon the latter. Finally, the patient's presumable personal conduct and cooperation constitute an important item in the inventory, because they may contribute to the final result, as failures on his part may have done already in favoring the advance of his disease to the stage in which we find it on examination.

It will therefore be seen that numerous factors must govern us in the selection of cases for successful treatment, whether this be specific or general, and that, with their increase, we may err in the conclusions based upon our analysis, although we follow the rule to study all evidence exhaustively, and although we endeavor to find at least a reasonable explanation for every symptom and sign in each particular case. The experience necessary to enable the physician to do this to the best advantage is always individual; it cannot be acquired from purely theoretical studies, but needs actual practise in the consultation-room and at the bedside, as well as the opportunity of checking practical observations in autopsies. Such experience, coupled with a keen interest and desire to ascertain the truth as far as it may be obtainable in our present stage of knowledge, brings the desired prognostic ability. Previous practical training under experienced guidance enables the student to develop his diagnostic and prognostic acumen sooner than will be the case if he is obliged to depend solely upon his personal study and upon the aid afforded by general and special textbooks and monographs.

THE RELATION OF TOXEMIA TO THE SYMPTOMS OF TUBERCULOSIS

The pallor of the skin and of the visible mucous membranes, which sometimes is sufficiently marked to attract attention, may constitute one of the earliest symptoms of tuberculosis. It is observed most frequently in children and young adults and has long since been looked upon as a suspicious omen, especially as it is found in girls; at the age of adolescence it was formerly regarded as a sign of "going into a decline." Blood-counts and hemoglobin-determinations usually give normal results, and when this is the case, the symptoms are due apparently to vasomotor constriction, and suggest the action of a toxin upon the sympathetic nervous system, while otherwise the direct action of the toxin upon the red cells may be responsible for the deficiency of their number and of the hemoglobin.

A like toxic origin offers, in our opinion, the most reasonable explanation for certain functional digestive disturbances which consist in lack of appetite or in its capricious character, in symptoms of dyspepsia, in an unsatisfactory gain in weight and growth in children, or in a gradual loss of weight even when the digestive organs are apparently not disturbed and when the appetite is sufficient for a normal amount of food to be ingested regularly.

The tired, languid appearance and the easily induced fatigue, disturbed sleep, the tired feeling on rising or after exertion, trophic anomalies of the skin, early decay of teeth, dilated pupils, headache, and other vague pains suggestive of neuralgia, myalgia or rheumatism, certain obstinate skin affections like acne or psoriasis, delay in the establishment of adolescence in boys and girls, and various other symptoms for which no adequate explanation offers, are in our judgment all phenomena of disturbed vasomotor function and suggest a toxic origin. We have long since assumed a like origin for certain functional disturbances of the heart, especially when they occur in the early stages of tuberculosis and are manifested by an unduly rapid pulse and by paroxysms of cardiac palpitation. The theory of the toxic origin of these symptoms is supported by the observation that symptomatic and general hygienic measures are

not followed by marked improvement. According to statements made frequently by our patients, therapeutic efforts had usually been applied and had failed before other symptoms appeared which pointed to the existence of tuberculous disease and brought them under our care.

Most of these symptoms have long been recognized as precursors of phthisis, but their treatment at our hands, under the most exacting application of dietetic and hygienic principles, including rest and regulation of exercise, and at times tonics and digestive aids as well as other appropriate remedies, was never followed by that degree of success which we are now accustomed to expect, and which we usually realize under specific treatment. We have every reason to believe that the development of a specific immunity is the important factor for the control and removal of these symptoms, and that they are in fact the manifestations of a specific toxemia.

We are of course unable to say which of the several possible toxic products of the tubercle bacillus is concerned in the production of these symptoms, and there is no reason why any of them could not cause toxemia, as we shall show when discussing their relation to fever; but as these symptoms usually appear at an early period, and often become manifest before a positive diagnosis of tuberculosis can be made, except with the aid of a tuberculin test, we are justified in considering the influence of the split products of a protein, or possibly even a toxin of non-proteid nature, which the tubercle bacillus elaborates in its living state; or then the action of a protein which becomes available after the death of the bacillus, and which, owing to its ready solubility, may be the first to appear in the local tissue and become available for absorption into the circulation.

In our opinion, the NIGHT-SWEATS which occur in pulmonary tuberculosis are often, if not always, associated with fever and usually have a like toxic origin. While the majority of patients with night-sweats belong to the advanced stage of tuberculosis, these may occur in early cases, when the functionating power of the regulating nerve-centers has become labile in consequence of intoxication and of other weakening influences.

The relation of fever to night-sweats became apparent in an investigation undertaken by one of us a number of years ago.[1]

[1] KARL V. RUCK; Journ of Tuberculosis, 1900, II, 166.

In the cases studied the night-sweats coincided, as a rule, with a rapid fall of the temperature, suggesting that the toxic irritation of both the heat- and vasomotor-centers subsided simultaneously during rest and sleep.

The infrequency of night-sweats in patients under specific treatment has attracted the notice of many clinical observers, and we have had occasion to call attention to it during our early experience with old tuberculin.

We have come to accept a like toxic origin for the colliquative diarrhea which is often present, especially in the terminal stages of phthisis, a similar cause being suggested in that it makes its appearance usually during the early morning hours or early in the forenoon, either in isolated attacks or in several evacuations which take place in rapid succession.

THE FEVER.—A study of the toxic symptoms, as they manifest themselves by fever, in the evolution of tuberculous disease of the lungs, when compared with other localizations, as for instance in lymph-glands, bones and joints, appears to us to show that these symptoms are more marked when the disease is localized in the respiratory organs, and that they are slight or absent more frequently in extra-pulmonary localizations. This may find an explanation in the blood-supply and in the respective functions of the organs or parts.

For many years the fever of phthisis has been considered as an important factor, if not the most important one, which is responsible for the emaciation and exhaustion of the consumptive; and it has, on that account, always been the object of special treatment, often with an entire disregard of the underlying cause or causes. The value of more direct remedies for the treatment of phthisis is to this day frequently estimated by their influence upon this symptom. Except for rest, as far as it is possible to impose it upon the diseased organ or upon the body as a whole, no mode of treatment directed against the fever has, however, been successful in modifying the course of phthisis.

After the introduction of antipyretics, it was hoped that their employment would be attended by the desired end and that the systematic and continued administration of these remedies would keep the patient free or nearly free from the fever and save him from its detrimental effects; it required only a short experi-

ence to make it evident that the consumption was not checked by the use of these drugs, but that the disease progressed in the same manner as before or even more rapidly. The expectation of controlling the fever by destroying the tubercle bacilli in the tissues or of reducing their pathogenic action, by the administration of germicidal remedies, also failed to be realized, because none of them could be given in sufficiently large doses without endangering the safety of the patient by their poisonous effects.

With the expectation that some degree of germicidal action would follow their use by long-continued administration in smaller doses, these remedies were employed persistently for many years, and they are still prescribed, especially in the form of creosote or of its derivatives which are the least objectionable of all. The effect of the external application of guaiacol upon the fever was indeed often striking, the application being followed within a few hours by a reduction of the temperature by several degrees; yet this use of the drug was discontinued on account of its effects which were only temporary, often disagreeable and even injurious, similar to those seen after large doses of coal-tar-antipyretics, which cause collapse-temperature, and because of the recurrence of the fever, accompanied by chills, after which it often reached higher maxima.

The creosote preparations continued to be prescribed in the absence of anything better, and their employment became so general that for years the drug was a routine prescription for tuberculous patients in any stage of the disease, as had formerly been codliver oil. Even the patients themselves became cognizant of its significance and inferred that they were being treated for tuberculosis when this knowledge had been withheld from them by their physicians. Such a general professional recognition of creosote could not have been possible had it not been for the fact of its modifying action upon the secretions of the mucous membranes, by which it often exerted a favorable influence upon the cough and expectoration, indirectly upon the fever, and especially upon gastro-intestinal disturbances.

The introduction of tuberculin raised hopes that its action would manifest itself also upon the fever; but these hopes were again disappointed in so far as they pertained to the advanced

stages of phthisis, while in the early, so-called closed stage, the excessive and frequent doses which were in vogue at first, and its failure to cause a complete immunizing response, account for the fact that a reduction of fever was noted only in a minority of cases with sufficient promptness to justify the assumption that the tuberculin treatment was responsible for the result.

The cause of the fever in tuberculous diseases has always been brought in relation with some pyrogenic agent which was supposed to be a product of the disease itself and which was believed to be absorbed from the lesions. Much study has been devoted to the nature and action of this agent. With the discovery of tuberculin, the administration of which gave rise to specific reactions including fever, the identification of the pyrogenic agent with the specific product of the tubercle bacillus appeared to be established beyond a reasonable doubt.

The reason why the administration of tuberculin in small doses caused general and febrile reactions only in tuberculous subjects, whereas much larger doses were ineffective in others, was not so clear. A satisfactory explanation was given in the further course of the studies in immunity, especially those of the mechanism of bacteriolysis, which has been described in Chapter IV. The theory postulates the activation of a latent function under the influence of specific stimulation, which follows the absorption of toxic products elaborated by bacteria after infection or after their parenteral introduction, and which manifests its action in the disintegration of the toxin or of the bacteria, through specific bacteriolytic substances in conjunction with a fitting enzyme or ferment. The specific substances which are spoken of as amboceptors vary quantitatively according to the frequency and intensity of the preceding stimulation, and consequently the degree of reduction of the related toxin to its simple elementary form is subject to like variations. In case the amboceptors are quantitatively insufficient, the reduction is incomplete and the partially reduced so-called split products have been found to cause toxic phenomena and irritation, especially upon the nerve-centers, which give rise to general and febrile reactions. Applying these observations to the occurrence of reactions to toxins derived from tubercle bacilli, in tuberculous or non-tuberculous subjects, we would expect fever to occur

in case the bacteriolytic function has been activated by previous infection with the bacillus of tuberculosis and by the absorption of an identical toxic product, provided the available specific amboceptors are quantitatively insufficient, and we would expect no febrile reactions in case these amboceptors were ample to reduce the amount of the injected toxin completely, whereas febrile reactions could not follow at all in non-tuberculous subjects because the bacteriolytic function has not been developed in them.

The occurrence or non-occurrence of fever in tuberculous subjects, after the administration or spontaneous absorption of toxic products of tubercle bacilli, depends therefore upon the existing balance between the amounts of specific toxins and specific bacteriolytic amboceptors that are present in the circulation. When the balance is in favor of the specific toxin, split products are formed and their amount and degree of reduction, also the time during which they are present in the arterial circulation, stand in relation to the toxic phenomena which follow.

The smaller the margin is in favor of the bacteriolytic amboceptor, the more readily will the balance turn in favor of the toxin and lead to the occurrence of split products. That this margin is often a very narrow one is shown by the slight causes which suffice to produce fever in the ordinary course of tuberculous disease, and by the minute doses of tuberculin which are required to produce febrile reactions.

The observation that such reactions become less marked or fail to occur, when the injection of the toxin is repeated, is to be expected if we are correct in our theory of these processes, since, in such a case, the function to reduce a given amount of toxin has increased because the cells respond more quickly, or because other more distant cells have been reached, that had not been influenced before by the toxin, and now contribute their quota of bacteriolytic amboceptors. The reduction is therefore more complete, or entirely so, and intermediary products do not reach the central nervous system in sufficient amounts, or they are not present in the circulation long enough to irritate the heat center enough to cause a rise in temperature.

A decided increase in dose is again followed by fever, for the same reason as this occurred in the first place, and in time

a still larger dose may fail in its turn to cause a reaction when the function has reached its maximal degree; but if thereafter an excess of the same toxin is absorbed or introduced, its reduction is again incomplete and slow; in consequence its intermediary products can accumulate anew, and enough of them may then appear once more in the circulation to produce fever.

Since the reactive phenomena must necessarily vary with the rapidity with which the foreign substance enters the blood, and also with the rate at which its intermediary products are eliminated by the excretory organs, it is to be expected that absorption from the diseased tissues is less rapid when the organism is completely at rest, as for instance during sleep, or when exercise is limited or avoided entirely, and when elimination is facilitated by the free action of kidneys, skin and bowels. A satisfactory explanation is thus at hand for the variations in the degrees of fever, or for its temporary absence as it is noted in the intermittent types.

It is, however, not necessary for the production of repeated or continued febrile reactions that all fractional parts of the toxin be absorbed or introduced in excess; the result will be the same as long as this is the case with one of them. The split or partially reduced products of a certain fraction of the bacillary toxin may also differ individually in their toxic or irritating action upon the nerve-centers, and the state of reduction may be of influence in the result.

We have reason to think in this connection more particularly of the lipoids of the tubercle bacillus, some of which we have found to be extremely toxic in animal experiments. They are the least soluble in the body-fluids, and become available particularly with the softening of caseous tubercle or with the advent of tubercle bacilli or of their fragments in the circulation. If we are correct in this respect, the relation of the lipoids or of their split products to the fever would be of additional interest, in view of the therapeutic results which have been claimed from the use of various drugs which are solvents for fats, like alcohol, ether, chloroform, and because the liberation and absorption of the lipoids in the organism might perhaps be favored by the administration of these agents at an earlier period, before the organism has acquired any or only traces of specific amboceptors for them. Our interest has not been di-

rected to the lipoids on account of the supposed value of alcohol or of other fat-solvents in the treatment of tuberculosis, but is based upon certain toxic and biological phenomena which we have observed repeatedly, and which may in time be explained otherwise. The point is of interest on account of the large use that has been, and is still being made of alcohol in the treatment of tuberculosis, often by experienced clinicians.

We find, therefore, that the fever in tuberculosis has its origin in the absorption of foreign substances which are produced by or constitute the specific products of tubercle bacilli, either of their secretion or metabolism, or of their body-constituents, and that it denotes a reaction between one or more of them and bacteriolytic amboceptors which are quantitatively insufficient to prevent the formation of split products, and their accumulation in the arterial blood. The increase of the specific bacteriolytic amboceptors is, however, limited and the absorption of toxic products may become increased to amounts through which an excess is present beyond that which can be neutralized by a maximal amount of specific amboceptors which the organism is capable of producing. This is likely to occur more particularly in acute miliary dissemination, or with the advent of caseous softening in pulmonary tuberculosis and when larger caseous areas break down rapidly. During each of these processes the absorption of specific toxin may occur continuously and in far greater amounts than the previously acquired defensive functions can compensate. The organism is then in a state of more or less constant intoxication and febrile reaction, for the removal of which it is necessary to prevent, or at least reduce and limit the absorption of the toxic products.

Clinically we seek to accomplish this by limiting exercise or by enforcing absolute rest, by immobilizing the lung as far as possible by strapping, or by its compression in the production of an artificial pneumothorax. In cases in which the destructive processes are extensive and multiple, or in which the fever reaches maxima of 101° F. and over, specific treatment, as a rule, is contra-indicated, because the absorption of tubercle bacilli and their products into the circulation is continuous and excessive. The enforcement of rest alone is inadequate, although it often moderates the fever, and the conditions for the efficient employment of the sovereign remedy of lung-compression, for

the purpose of checking the absorption, are not often given in the advanced stage of phthisis.

The theory that the lack of specific amboceptors for one or more of the bacillary products is responsible for the occurrence of split products which maintain the fever, and that the administration of their related antigens, needed to produce the lacking amboceptors, would enable the organism to complete the reduction, has had our attention, and we have made attempts of studying its correctness in a number of cases. So far we have been able to influence the course of the fever in phthisis, by the administration of partial antigens, in only a few instances in which it was possible to show amboceptors for one or more of them and not for others. As a rule, we found no specific amboceptors at all in the sera of patients whose temperature was high; more often we found that such sera appeared to remove all specific amboceptors from immune sera of known titer against which they were tested, especially in cases in which tubercle bacilli were present in the blood. Our experience is, as yet, not sufficient to justify us in abandoning trials in this direction, or in affirming or rejecting the theory itself; all that we can say is that, so far, we have not met with the successes which the theory would justify us to expect, and that usually we did not find it applicable in actual practise.

The evident relation of the fever to an insufficiency of specific bacteriolytic amboceptors suggests the question whether it might be possible to increase them, as is done in the administration of specific antitoxins, by the therapeutic injection of a serum from an actively immunized animal in which their presence has been demonstrated in maximal amounts.

Attempts to use immune sera therapeutically, in order to transmit an actively acquired immunity to another organism, which constitutes a *passive immunisation* for the latter, were undertaken at first rather empirically and, later, on similar principles and with like purposes, as antitoxic sera are used, viz., in the expectation of supplying artificially the desired bactericidal antibodies. They are among the earliest efforts to transfer resistance from those in health or from those who were actually immune, to others who suffered from an infectious disease. At first the normal sera of dogs, goats, horses and of other animals were used experimentally and therapeutically for this purpose, and later the sera from animals after these had been treated with the related bacteria or with their supposed toxic products. It was particularly in tuberculosis that

these efforts were numerous. We remind the student of the early work, especially of French observers, in the use of normal sera from supposedly resistant animals, and the employment of antituberculous sera from treated animals, which latter was brought to the attention of the medical profession particularly by Maragliano of Genoa, and by Marmorek of Paris.

Some of the earliest experiments in this direction were reported by Héricourt & Richet,[2] with the blood and the serum of dogs. At the second and third French tuberculosis congresses experimental and clinical results were submitted by Héricourt, Semmola, E. Vidal, Pinard & Kirmisson, S. Bernheim, Babes and others, on experiences especially with hypodermic injections of dog-serum, and with injections or transfusions of goat's blood.

Deutschmann[3] employs a serum which he obtains from rabbits after feeding them with gradually increasing doses of yeast, and claims that it is of value in local and general parasitic infections, including tuberculosis. Dr. Gimbert of Cannes prepared a serum from cow's milk, which was recommended by Lerebouillet[4] before the French Academy of Medicine, for use in diseases that are characterized by malnutrition and debility, especially in cases of pulmonary phthisis which had not advanced too far.

Magnant[5] proposed the treatment of phthisis by subcutaneous injections of serum taken from blisters raised upon the skin of consumptive patients. Gilbert[6] advocated an *autoserotherapy* by employing pleural exudates for injections in the same patients, claiming that antibodies are transmitted into the circulation in this manner, while they are not absorbed by the inflamed pleura. Under the name of *heteroserotherapy*, Jousset[7] recommended the injection of "the serum of other patients, which is defibrinated and heated to 55° C.," in amounts of 20 to 50 cubic centimeters. He claimed that the procedure of Gilbert was not without danger; but this was employed, nevertheless, by many clinicians with apparently beneficial results.

Attempts in passive immunization against tuberculosis were made in this country by Trudeau & Baldwin, de Schweinitz, Paquin, Fisch, with sera from animals, taken after treatment with products of tubercle bacilli, but without sufficient results to warrant their continued practical use. Like efforts were made abroad by numerous investigators, especially by Maragliano[8] and later by Marmorek,[9] as mentioned above, and the immune sera

[2] HÉRICOURT & RICHET, in VERNEUIL; Études expérim. et clin. sur la Tuberculose. Paris, 1880-90, II, 381; 1892, III, 139.

[3] R. DEUTSCHMANN; abstr. Centrlbl. f. Bakt. Ref. 1908, XLI, 581.

[4] LEREBOUILLET; Bull. Acad. d. Méd. d. Paris; 3e sér., 1899, XLII, 96.

[5] MAGNANT; Bull., Acad. d. Méd. d. Paris; 3e sér.; 1897, XXXVIII, 354.

[6] V. GILBERT; Rev. méd. d. l. Suisse romande; 1910, p. 24.—Abstr. Fol. Serol.; 1911, VII, 99.

[7] A. JOUSSET; abstr. Zeitschr. f. Tub. 1912, XIX, 296.

[8] E. MARAGLIANO; Berl. klin. Woch.; 1895, XXXII, 689.—Ann. Istit. Maragliano, 1909, III, 196; cf. Centrlbl. f. Bakt., 1909, XLV, 316; and elsewhere.

[9] A. MARMOREK; Zeitsch. f. Tuberkulose; 1900, I, 444.—Berl. klin. Woch.; 1903, XL, 1108; and elsewhere.

of these authors have been employed clinically in large numbers of cases over a period of years. More recently, Bruschettini [10] and Ruppel & Rickmann [11] introduced sera for passive immunization, but Bruschettini supplements the action of his immune serum by an active immunization, and the "Höchst" antituberculosis serum, reported upon by Ruppel & Rickmann, has never found practical application.

In so far as one can judge from published case reports, neither these nor various other sera have been effective in influencing the course of the fever in phthisis in a decided manner, although good clinical results have been observed frequently during their administration, especially in slightly febrile or in afebrile cases.

In our own experimental studies, in 1897, with serum from goats, taken after prolonged treatment with Tuberculin R. and with Watery Extract of Tubercle Bacilli, we were successful in so far as by adding a like quantity of the immune serum to 0.5 cc. of tuberculin (i.e., the minimum dose that kills tuberculous guinea-pigs in forty-eight hours), and injecting this mixture into tuberculous animals, these did not succumb, whereas the controls all died within forty-eight hours. The treatment of tuberculous patients with this serum was not followed by apparent benefit, and the results from active immunization were much more satisfactory. As we considered febrile cases of phthisis to be unsuitable for active immunization, the administration of the serum was then limited to this class of patients, without any apparent amelioration of the course of the disease, either in regard to the fever or to the other symptoms, and its use was finally abandoned because of occasional accidents which we now recognize as having been due to serum anaphylaxis.

In our attitude toward passive immunization in the treatment of tuberculosis, we have been guided by the consideration of the chronic nature of the disease, in which we believed it to be of importance that the organism should possess an active immunity, and be enabled thereby to defend itself against the tubercle bacillus and its toxins. We reserved our trials with

[10] A. BRUSCHETTINI; Trans. Internat. Tuberculosis Conference; Brussels, 1910, p. 314.—VII. Internat. Congr. of Tub.; Rome, 1912 (Repr.).

[11] W. G. RUPPEL & W. RICKMANN; Zeitschr. f. Immun. frsch. 1910, VI, 344.

immune sera, therefore, to cases in which the active method appeared to be contra-indicated, as we endeavored to show in our discussion of the selection of cases. We find in more recent years that many other clinicians appear to have adopted a similar attitude, which has led to the gradual abandonment of passive immunization in favor of the active method. In this connection it is of particular interest to note that Maragliano [13] no longer depends upon his immune serum alone in the immunization against and the treatment of tuberculosis; while he still administers his serum in clinical cases, as a preliminary course, thereafter he employs and depends for his final results upon the active method by administering the bacillary body-constituents in the form of their extractives. A similar change of attitude is noticeable in the use of antisera for the treatment of so-called mixed infections in febrile phthisis, the active method in the form of autogenous or stock vaccines having replaced the passive method almost if not entirely.

We have pointed out that, in so far as it is subject to demonstration by means of the complement-fixation reaction, bacteriolytic immunity appears to be limited quantitatively, in that apparently it cannot be increased by further treatment after a certain amount of specific amboceptors has been produced. In this respect the production of bacteriolytic immunity differs essentially from that of antitoxic immunity in which the titer of the antitoxic serum can be made to reach a very high degree. Under these circumstances the doses of the bacteriolytic serum, required for treatment, would need to be adjusted to the amount of bacillary toxins that are present in the blood, and this would necessitate very large doses in certain cases.

If we are correct in our observation that the fitting ferment, which is necessary for the bacteriolytic action of a tuberculosis immune serum to become manifest, is specific, it would also be necessary that the immune serum be administered in its fresh active state. In any case the necessary dose would need to be repeated perhaps daily, and the treatment would have to be continued until the excessive absorption of the bacillary products no longer occurred, which may be deferred for many months or indefinitely.

Further, we would have to reckon with the occurrence of anaphylaxis due to the repeated administration of a foreign serum, and with the possibility that the artificial increase of the bacteriolytic power of the blood, beyond that which the organism itself can produce, is not indifferent, and that similar results may be experienced from the rapid reduction of large amounts of specific toxins, as occur when a foreign serum is reduced rapidly, in consequence of which the patient may be poisoned in a similar manner.

We are at a loss to account otherwise for our observation of deaths in highly immunized animals, which followed within a few days after their infection, and for like fatal results in bactericidal experiments after the intraperitoneal infection of normal animals with the contents of test-tubes

[13] E. MARAGLIANO; *loc. cit.*, Centrlbl. f. Bakt. Ref. 1909, XLV, 316.

in which virulent tubercle bacilli had been subjected to the bacteriolytic action of fresh immune serum.

We find, therefore, that formidable if not insurmountable difficulties are likely to be met with in attempts of preventing the toxic fever in the course of phthisis, by trying to supply the necessary specific antibodies artificially. In recent attempts on our part to do this we administered comparatively large doses of inactivated immune serum of maximal amboceptor-content, as determined by the complement-fixation test, without observing any appreciable influence upon the fever, except in one case in which the temperature was reduced temporarily after the first, and again after the third intravenous dose. After the intravenous administration of the fourth dose of 15 cc. given six days later, decided anaphylactic symptoms followed immediately; together with the entirely negative results in other cases, this accident caused us to abstain from making additional trials. Under the circumstances it was not entirely clear whether the symptoms of anaphylaxis were caused by the foreign serum, or whether they stood in relation to the reaction between the specific components of the immune serum and the toxic products of tubercle bacilli which, in this case, was well possible because tubercle bacilli were found in the blood of the patient before each dose was administered. We are inclined, however, to consider the foreign serum as standing in relation.

From the foregoing considerations concerning the fever as a toxic manifestation in tuberculosis, it follows that we are practically without an efficient remedy for its successful treatment, at the present time, in cases in which the specific toxins of the tubercle bacillus are absorbed in excessive amounts, except in the comparatively infrequent instances in which a sufficient degree of lung-compression can be accomplished, by which their further absorption may be arrested.

In the light of our studies of the subject, we must expect that most cases of this kind will succumb to their disease. Our observations give us a satisfactory explanation also for the fact that even a maximal degree of immunity, as we are now able to induce it by specific treatment, can fail in cases in which it has not developed before a period when larger foci and aggregates of tubercles have caseated, and why the acquired im-

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munity cannot afford a guarantee for the future in such cases, until the caseous tissues have undergone encapsulation so firmly, completely and permanently that they are no longer subject to softening and absorption regardless of incidental exciting causes, such as acute respiratory diseases, trauma, etc., which can initiate such a change.

While we expect to make trials with immune sera in their active state, with a view of studying their action upon the course of febrile phthisis, we shall consider likewise the prospect of success from efforts leading to the increase of the bacteriolytic action of the patient's own serum to its maximal degree, in case the inferences are correct which we draw from our observations mentioned in Chapter IV, on p. 94: namely, that the bacteriolytic action of immune serum in tuberculosis depends upon the presence of a specific thermolabile substance of the nature of a ferment, and that this substance forms in response to immunization with a certain fraction of the bacillary body-substances, to which is attached a similar toxic substance, also of ferment-nature, which causes the development of a specific bacteriolysin.

This assumption has the support of certain observations in the production of antitoxic immune sera, and of other evidence. In antitoxic immunity the specific toxins, and the antitoxins which are formed in response to their administration, are both believed to be of the nature of ferments. Like other ferment-actions, their action can be destroyed by heat, and they deteriorate spontaneously on standing. The toxin is believed to be a product of bacterial secretion peculiar to a small number of bacteria; the antitoxin being itself of ferment-nature, it does not require the cooperation of another ferment for the neutralization of its related toxin; it is capable of being increased enormously by the administration of the toxin.

The bacterial body-substances or endotoxins of tubercle bacilli, on the other hand, are supposed to have no ferment-action, to be thermostable, and to be of proteid- or of lipoid-nature. The bacteriolytic amboceptors, which appear in response to the administration of the endotoxins, are also thermostable, they are not of ferment-nature, and the cooperation of another ferment is required for their action.

It will be observed in antitoxic immunity that the specific toxin and the specific antitoxin are both of ferment-nature; the reaction occurs therefore between two ferments. While the correctness of these propositions appears to be established and accepted without a reasonable doubt, it may nevertheless be permissible to inquire whether there are no exceptions to these observations and whether all bacterial body-substances or endo-

toxins, including those of the tubercle bacillus, comply with them. This inquiry appears justified if we remember that the production of so-called true toxins is limited to only a few bacteria and that they constitute an apparent exception to all others.

Knowing that all cells, whether of animal or vegetable origin, contain ferments, and finding that the results of more recent investigations of their action indicate that they are specific for each kind of cell, we have no reason to assume that the tubercle bacillus-cell does not also contain a ferment, and some reason for the belief that it may be specific. This ferment need not be present in sufficiently large amounts to betray itself by manifesting its action readily; it may be bound intimately to one or more of the constituents of the cell-body and may thus escape detection until the body-substances themselves are brought into solution before being exposed to temperature- or other influences which destroy it or cause its spontaneous deterioration.

In formulating a theoretical basis for our further studies, we assume that the tubercle bacillus-cell contains a ferment, and that the parenteral introduction of this ferment produces an antiferment, the same as we find it to occur in response to the administration of the true toxin, in the form of an antitoxin.

Our studies and observations caused us to search for the manifestation of the ferment particularly in one of the lipoids, because we have found it exceptionally toxic, and in our studies of the immunizing action of the several proteins and lipoids of the tubercle bacillus, we succeeded in protecting animals with this same highly toxic lipoid (p. 290). We also found marked autolytic changes in our vaccine, which contains this lipoid in a very minute amount, when it was kept for a number of months at room-temperature, and we found further that other preparations, which have been heated in the course of their manufacture, remain unimpaired for years without any precautions in regard to temperature-conditions.

The opinion expressed by Koch, that living tubercle bacilli were necessary for a complete antibacterial immunity in tuberculosis, and the success in immunization of cattle with living tubercle bacilli of the human type, may possibly find their true explanation by the presence of this specific ferment. Our observations in bacteriolytic studies *in vitro*, and those of a certain variability of the degree of morphological changes under the use of active sera which showed little difference in their amboceptor-contents, suggest that the germicidal action of immune sera depends upon a specific ferment-action, and likewise that the complement-binding amboceptors need have no part in the destruction of virulence.

THE FEVER IN ASSOCIATED INFECTIONS.—A toxemia due to other bacteria may have a similar origin and mechanism of action in causing fever, as we have accepted it for the toxic products derived from the tubercle bacillus; it may then be

solely responsible for, or may participate in causing the temperature-rises of the open stage of phthisis.

The associated or so-called MIXED INFECTIONS have been the subject of much study, resulting in widely differing opinions which remain unreconciled to this day. There can, however, be no doubt in regard to their importance and to their frequent relation to the fever in the course of phthisis. Some years ago the pus-cocci, especially the strepto-types, were supposed to be responsible for the most frequent and serious associated infections. In our experience we feel justified in assigning a greater share to the pneumococci and to the influenza bacillus as exciting causes of destructive changes in the closed stage, and as responsible for fever and for inflammatory complications which may occur in the open stage.

Theoretically we may expect like reactions to the toxic products of these microörganisms, as they are observed from those of the tubercle bacillus, and we may also expect that suitable specific vaccines will produce an immunity against them. The fact that, in the course of such associated infections, the patient often fails to acquire the necessary degree of immunity spontaneously, but that it develops frequently after the administration of a specific bacterial vaccine, appears to depend on similar conditions as they are found in tuberculosis: namely, on the condition that the bacterial body-substances are necessary for the development of bacteriolytic immunity. We have often been able to bring about the prompt subsidence of fever by means of autogenous bacterial vaccines, and we have also observed unmistakable focal reactions in the lungs, especially in cases of pneumococcus infection. Like in tuberculosis, focal reactions appear to indicate that a limited amount of specific amboceptors against the related bacteria or their toxins were present in the reacting tissues and it was made evident by the eventual disappearance of focal reactions that they were capable of increase by further specific stimulation.

In this connection we wish to mention another interesting observation, namely, that during the acute period of an associated infection the specific amboceptors for tubercle bacilli, which previously had been present, as a rule could not be demonstrated in the sera of the patients, but that they reappeared afterwards spontaneously. We have made like observations in regard

to specific agglutinins, the titer of which fell steadily and rapidly, sometimes to zero, and returned later to its former degree. We are without an adequate explanation for these observations, but they remind us of our similar experiences with associated infections in guinea-pigs, in the course of experimental studies, as will appear later in their consideration. Another point of common interest is that these associated infections are at times as seriously detrimental in the human subject as we have found them in our experiment animals, and that the cause for the loss of specific protective substances may be the same.

CHAPTER IX

THE SPECIFIC TREATMENT OF PULMONARY TUBERCULOSIS (*Continued*)

THE ADMINISTRATION OF SPECIFIC REMEDIES

The several specific remedies for the treatment of tuberculosis contain either glycerin or a small amount of phenol for their preservation; our preparations are protected against air-infection by the addition of 0.4 per cent of phenol. In hot weather, or when they are not to be used within a few months, all these preparations should be kept in a cool place, preferably in the refrigerator, and this precaution is called for particularly for the prolonged keeping of our vaccine, if its power to cause reactions is to be preserved in its full degree.

In the examination of our results of prophylactic vaccination, in Chapter VII, we showed that a complete bactericidal immunity can be demonstrated in suitable cases, by laboratory tests and by animal experiments, after the administration of one full dose of our vaccine. For therapeutic application, we usually begin with comparatively small doses and endeavor to accomplish the same result gradually; yet it has often been possible to show a maximal amount of specific antibodies in the course of the first month of treatment, and in some cases long before the well-marked open or closed tuberculous lesions, existing in the lungs or elsewhere, had healed or disappeared.

The object of giving small initial doses is to ascertain the sensitiveness of the individual patient, which varies with the character and extent of the tuberculous changes, and with the degrees of spontaneously acquired immunity against one or more of the specific toxins contained in the remedy that has been selected. When the real purpose of the small initial doses has been accomplished, by establishing the degree of sensitiveness to the bacterial remedy in an individual case, we believe that the treatment should be continued with amounts that are large

enough to produce distinct but not excessive focal reactions, as will be discussed in detail in the following pages. We are convinced that we have often lost valuable time, in the past, by giving too minute doses and by increasing them too slowly in the course of specific treatment. For several years prior to the therapeutic use of our vaccine, we gave 0.1 cc. of the 10 per cent solution of the Watery Extract of Tubercle Bacilli as a beginning dose; our increasing experience has led us to give, as a rule, 0.1 cc. of the full strength of both preparations, the Watery Extract and the Vaccine, for the first or trial-dose.

For the therapeutic use of tuberculin preparations or of tubercle bacillus-emulsions, the initial doses, administered by different observers, vary greatly in amount; clinicians who endeavor to give the doses small enough and to increase them slowly enough to avoid reactions of any kind, inject initial doses of one millionth or less of one milligram. In our experience with old tuberculin, we found that an initial dose of 0.1 mgr.. that is, 0.1 cc. of a one-tenth per cent solution, rarely produced reactions, and we believe that 0.01 mgr. of tubercle bacilli in emulsion may be administered without any danger of causing necrosis at the site of its subcutaneous injection.

If a dilution is desired for the purpose of measuring small initial doses accurately, it may be made by mixing one part of the remedy with nine parts of boiled water to which 0.4 per cent of carbolic acid are added, and further dilutions are made in the same manner; they may be preserved in a sterilized vial. It is of course understood that, in making the injections, the usual aseptic precautions are to be observed.

We have not found that it is necessary to adjust the doses for children as we would in the administration of drugs and, although we find that the maximal single doses need not be as large as in adults, the doses stand less in relation to age and body-weight in producing reactions than they do to the extent and character of the tuberculous lesions and to the specific antibodies which have already been acquired.

We can therefore be governed by the same factors in the size of our beginning therapeutic dose for children and for adults, and we administer the same initial doses, except in very young children and in infants, in whom we make the first trial with a dose about one-tenth of the usual size.

No routine scheme can be outlined for the size of the doses, or for their increase and the frequency of administration. If, as we believe, the doses must be sufficiently large that the tuberculous organism can respond to them with a focal reaction, in order to obtain the best clinical results, it is necessary to adjust and increase them with care for each individual case. If, however, it is desired to prevent reactions entirely, the course of some clinicians may be followed who begin with a millionth or even less of one milligram of the specific remedy, and increase these minute doses until their uselessness has been demonstrated or until, in the meanwhile, they have increased sufficiently to cause reactions. This course requires no selection of cases; but it must be unsatisfactory, as it is apt to lead to disappointment and confusion, and is attended by what may prove a serious loss of time.

In respect to the frequency of administration, we have elsewhere (p. 131) explained our reasons for the interval of five days which we observe, unless general or focal reactions have occurred which have not subsided twenty-four hours after the administration of the remedy. In this event, we give the next dose five days after the subsidence of the signs of reactions. Some clinicians administer the specific remedy at shorter intervals, or daily, especially when the doses are given so small that no reactions occur. As a rule, we have adopted longer intervals than five days only when large doses were found necessary to cause focal reactions in lesions which persisted after all others had disappeared or had reached a state of healing and fibrosis. By waiting several weeks or even longer, we believe to have been able to produce focal reactions with doses which, on more frequent repetition, had apparently become ineffective for this purpose.

After the first or a subsequent dose has failed to cause a reaction, an increased amount becomes necessary, the increase depending upon the degree of the reactions which have been observed. Unless the preceding reaction has been severe, we usually increase our doses by at least 0.1 cc. of the full strength of our preparations.

In connection with the therapeutic administration of specific remedies, the question of how long this shall be continued may properly be considered in this place. We have frequently been

asked for information in this respect, also for our opinion in regard to the size of the final maximal dose which should be given and to the total amount of the specific remedy required for a given case; such questions are quite natural on the part of physicians who have no personal experience. While it was impossible to answer them definitely, our position can be inferred from the discussion of specific reactions, which follows. A perusal of individual case-histories reported on p. 187, in connection with prophylactic vaccination, and of the case of a patient mentioned on p. 268, will give additional information. It will be observed that the patient, whom we dismissed as apparently recovered, was in the early stage of phthisis, having a small cavity in one lung, a tuberculous infiltration in the other, and also a tuberculous complication of the larynx. He was treated by us a little more than two months. During this time he received 14 doses of vaccine, of 0.2 cc. to 1.5 cc. each. or a total amount of 6.6 cc., and he did not react to the maximal doses of 1.5 cc., nor to the preceding dose of 1 cubic centimeter. Judging from our experience with other similar cases, this is about as short a time, and the amount of vaccine used was as small as we could expect. The treatment of this patient would have been prolonged materially if we had followed a routine in the administration of the vaccine and if we had given the doses with a view of avoiding reactions.

Patients are, however, met with who react to very small doses. We have observed instances in which so small a dose as 0.01 cc. or even less than that caused marked general and focal reactions, and other physicians have called our attention to similar experiences on their part. As far as we now know, these experiences are exceptional and the reactions proved beneficial rather than harmful. We advise those who wish to be assured absolutely that no severe reactions will follow upon the administration of the first therapeutic dose of the vaccine, to use dilutions, as indicated heretofore, and to make a preliminary trial by giving 0.1 cc. of one or the other of these dilutions; the doses can then be increased according to the results observed.

More time is often necessary in more advanced cases in which the pathological changes are more extensive, or in which complications are present, and the number of doses required, as well as the total amount of vaccine used, is correspondingly

greater. We believe, however, that non-effective doses are often given in instances in which their action is not or cannot be observed accurately, or because the site of the subcutaneous injection is not varied.

Some years ago we attempted to secure a more efficient response to the vaccine by dividing the individual doses into smaller portions and injecting the fractional doses at the same time in different parts of the body, with a view of obtaining a better response. This mode of administration failed to prove of advantage and was given up.

As to the most suitable localities for the injections, we alternate between the extremities, and also inject into the lower quadrangles of the abdomen, occasionally into the loose tissue on the chest or back.

When a dose fails to cause local reactions at a new site of subcutaneous injection, it may be increased decidedly and to advantage; after larger doses have been reached without causing focal or general reactions, they may usually be increased by amounts of from 0.2 to 0.5 cc. It has not been often, under the precautions which we observe, that we were obliged to exceed 1.5 to 2.0 cc. of vaccine for a single subcutaneous injection; when such large doses were required, it was for the purpose of causing focal reactions in old, more or less walled-off cavities which refused to heal, or in extrapulmonary localizations in which it was not possible to inject the vaccine directly into the peripheral tissues of the lesions.

In cases of open phthisis it will occur that, although the specific treatment has been long continued and large doses have been reached, no further improvement will be observed in the physical signs which are still present, especially in râles and rhonchi which may be confined to a cavity or are heard in other parts of the lungs. If in afebrile patients of this class tubercle bacilli continue to be present in the expectorations, and large doses of the specific remedy cause no focal reactions, we sometimes resort to its intravenous administration, for which we employ about one-third of that subcutaneous dose which had failed to cause a reaction; and then we increase or repeat the doses according to the non-occurrence or occurrence of focal and general reactions. So far, we have not increased the intravenous dose over the subcutaneous one which had failed to

cause a response, and in case focal reactions occurred at all, we noted them, as well as further improvement in response to doses which were considerably smaller.

Failing to cause reactions by the intravenous method of administration, we have but one procedure left which offers a prospect of success, namely, the direct injection of the remedy into the lung lesion itself, the same as we and others have done with marked benefit in accessible extrapulmonary tuberculous localizations. Although we have not resorted to the intrapulmonary injection of specific remedies in many cases, and are therefore not prepared to express an opinion of its advantage, we are encouraged and intend to make further trials, in the future, when suitable cases come to our notice. We deem these trials proper and justifiable, because they are devoid of danger under aseptic precautions. Having already learned that intrafocal injections can cause marked focal reactions in very small doses, we believe that, in cases in which no reactions occur after full doses on subcutaneous or intravenous administration, the first intrapulmonary dose should not exceed 0.1 cc. of our vaccine.

In similar cases in which we fail in accomplishing further improvement and the subject of an associated infection has not before been investigated thoroughly, this should now be taken up, even though there may be little or no fever. Other pathogenic microörganisms are capable of maintaining secretions in pulmonary cavities and in the bronchi, and of causing chronic peribronchial and interstitial changes which may not be attended by fever; these changes improve or subside under the use of autogenous or stock vaccines, providing these are actually specific in that the bacteria responsible for the mixed infections are present in the vaccine in sufficient amounts. We may obtain the necessary diagnostic information in this respect by careful observation of the reactions which follow.

In case the vaccine contains the microörganism, which is responsible for the clinical signs and symptoms, in sufficient amount, it will cause reactions similar to those observed in tuberculous lesions after the administration of specific products of tubercle bacilli. We shall consider these reactions further on, and we believe them to be equally essential for their therapeutic effect and to be of like diagnostic import. The observation of

specific reactions puts us in a position to avoid prolonged and useless treatment in cases in which the vaccines have no specific relation and therefore do not produce reactions; whereas, if they do occur, we are governed by them in the size of doses and in the frequency of administration. Failing to produce focal reactions with a comparatively large dose of a given vaccine, we discontinue its administration and proceed with further efforts, with a view of obtaining the related bacteria by culturing and by microscopic studies of the sputum. In this manner we have met with success on subsequent trials with sufficient frequency to justify their repetition.

SPECIFIC REACTIONS

What we shall have to say of reactions applies to those occurring in response to any of the specific products of the tubercle bacillus, which include therefore all preparations of tuberculin and all emulsions and solutions of tubercle bacilli. If, here as elsewhere, we find it convenient in our discussions to speak of one or the other of our preparations, it is because our personal observations were made in greater part in the course of their administration.

It has long been known that normal, that is to say, non-tuberculous, subjects do not react to tuberculin, even when it is administered in large doses, and we find the same to be true in the case of our vaccine. A dose of one cubic centimeter of the latter, which we gave subcutaneously to an infant two months old, without a history of exposure to infection and negative to the v. Pirquet test, caused no general disturbance and no local reaction at the point of injection. While this dose was unnecessarily large, we have no doubt that even larger single doses may be injected without giving rise to reactions of any kind in the normal organism. The object of giving the large doses of vaccine in this and in other cases, was to determine whether the antibodies formed in the blood-serum of non-tuberculous subjects, in response to vaccination, would persist for longer periods of time than they do after small doses. We recall, in this connection, as of historical interest, that Schreiber[1] injected as

[1] SCHREIBER; Deut. med. Woch. 1891, XVII, 306.

much as 5 cg. of old tuberculin in single doses, in newborn babies, without causing reactions of any kind.

Tuberculous individuals, on the other hand, react locally and generally, unless the infection is of so recent occurrence that no specific bacillary toxins, or not enough, have been absorbed into the circulation, or, unless in a later period, a sufficient degree of general immunity has been acquired to neutralize the effect of the amount of toxin contained in the dose, by causing its complete reduction either locally or after absorption into the circulation. We have also found in very early cases that reactions occur to tuberculin, or to similar preparations which are chiefly or entirely secretion- or metabolic-products of the tubercle bacillus, at a time when certain of the body-extractives do not yet cause reactions.

We have on previous occasions referred to our experience as indicating a definite relation between focal reactions and general and local improvement. In patients with slighter degrees of pulmonary disease, the general improvement and the retrogressive changes in the tuberculous lesions, after a few doses of vaccine, are often of such a nature that they can be accepted as sufficient to establish an apparent cure, although no marked focal reactions occurred. We have noted, however, that the physical signs in these cases did not always disappear as completely as we have observed frequently in other cases in which marked focal reactions had made their appearance. It seems to us that in slighter lesions the tendency to fibrosis was greater without the occurrence of focal reactions, whereas, when focal reactions were observed, the percussion-note as well as the auscultatory signs changed rapidly and became normal or nearly so.

In cases in which the lesions were more advanced or had reached the destructive stage, in which general and local improvement had become unmistakable, and the sera of the patients had given satisfactory evidence of the acquirement of a complete immunity and had proved germicidal *in vitro*, we noted that, unless the specific treatment was continued, the local improvement ceased even though the subjective symptoms had disappeared and the general health of the patients seemed to be restored. On resuming the specific treatment, after weeks or months, we often found that no material change had taken place in the physical signs, in the interim, but a further retro-

gression became evident when the treatment was continued with increasing doses. This was the case particularly if one or several of the doses were followed by focal reactions. Without the occurrence of focal reactions, the local changes for the better often developed so slowly that it required several months to bring about a marked difference; in secreting cavities, we frequently missed any advance toward their final healing, even after prolonged administration of the remedy during which the doses had gradually been increased to one cubic centimeter and more. Such a slow progress often changed, sometimes quite abruptly, in instances in which a marked focal reaction followed upon a decided increase of the dose. It would be easy to illustrate these experiences by many detailed case records and to meet the possible objection that we were misled by coincidences, by hundreds of like observations upon tuberculous lesions of the upper air passages and of other localities which are subject to direct inspection.

In case a reaction occurs at all to any dose, it is always observed at the site of the subcutaneous injection, if not to the first, then to an increased dose of the specific remedy. In the absence of a local reaction, we should seriously doubt the relation of fever or of a supposed focal reaction to the dose. In cases in which local reactions are accompanied only by slight rises of temperature, it is possible that no reactions will be observed in tuberculous lesions in the lungs or elsewhere; but while these may occur without an increase of temperature, local reactions are always present.

With reference to the favorable influence of focal reactions, we may add that, in our opinion, they cause an increase of the local immunity in the tuberculous tissues and in their periphery, and that this is produced through the active hyperemia by which the specific stimulation becomes manifest and in consequence of which the toxic bacillary products are absorbed more rapidly.

The lack of vascularity of tubercle, the dense barrier to rapid exchanges between the arterial blood and the tissue-fluids in fibrocaseous lesions, or in lesions which are more or less encapsulated, can readily explain the accumulation of toxic products in tuberculous lesions in which no free amboceptors are available, because the local cell-receptors are occupied with toxin-

molecules, and most if not all of the amboceptors, which are conveyed to the lesion with the blood, become bound by other free toxin-molecules. With the occurrence of an active hyperemia, the conditions are changed, the accumulated toxin is absorbed more freely and rapidly, a greater number of bacteriolytic amboceptors, formed by the local tissue-cells, become available and they are more largely augmented by others that are present in the blood which gains access more freely while the hyperemia lasts. The removal of specific toxins or the better access of specific amboceptors to the lesions, or both, may thus stand in relation to the beneficial effects which we have observed from focal reactions.

For the information of those who have little or no experience in the observation of focal reactions, we may add that their anatomical appearance, in the larynx or in other visible lesions. in patients under treatment or as observed in the autopsy of experiment animals, consists in a vascular turgescence or in a congestion which is attended by more or less swelling and is sharply defined, being limited to the tuberculous focus or to its immediate periphery, or both. In other tuberculous lesions, that are not subject to direct inspection, our evidence is of course circumstantial, but nevertheless valuable. Objective symptoms of a focal reaction in the lung consist in a temporary increase of the cough and of expectoration, and tubercle bacilli may appear in the latter when they had not been observed previously, or there may be a decided numerical increase.

In pulmonary lesions, auscultation can readily determine an increase of the local phenomena, provided the reaction occurs in a locality that was not previously the seat of moist râles and rhonchi, and provided also that an exact record of former findings, made before the dose was given, is available for comparison. It is important to bear in mind that the reaction is not to be expected throughout the entire tuberculous territory, and that the pulmonary lesions which react first and most readily, as a rule appear to be those in which the physical signs are least marked, which may therefore be presumed to contain more recent deposits of tubercles, and hence to be most accessible to the circulation. An increase of the auscultatory signs indicates that the area over which it can be recognized is reacting, and the inference becomes clinically conclusive if these in-

creased signs have become less or if they have disappeared twenty-four or forty-eight hours later, especially if this cycle has been observed repeatedly.

The signs of focal reactions may appear also over lung-areas which have been passed for normal before the administration of a specific remedy, probably because the pathological alterations were not yet sufficiently marked; the reaction then aids in their recognition. It is therefore always advisable to examine both lungs in their entire extent and to include the findings in the record of examinations. Recent tubercles are often present in areas adjacent to those in which marked physical signs are found, and they are likely to react first; the soft vesicular character of the inspiration then becomes rough or slightly harsh, sometimes weaker; and a suggestion of stickiness, or even of fine crepitations, may appear. If these are not heard during normal breathing, they can sometimes be brought out if the patient coughs and immediately thereafter takes a rapid deep inspiration. Reactions may occur also in the pleuræ and manifest themselves by pleural crepitation or friction, with or without subjective signs of pain or soreness.

The results of examination, with a view of detecting reactions in the lungs, may leave us in doubt in cases in which the reactions are very slight and also in advanced cases of phthisis in which a diffused bronchial catarrh is present, the signs of which may obscure those due to the reaction. In the former instances we repeat the dose before we increase it, and, if in cases with extensive catarrhal signs we are unable to satisfy ourselves sufficiently of the presence or absence of focal reactions by observing changes in physical signs, we increase the doses slowly and continue our examinations especially over outlying areas which may be free from catarrhal signs, and we also watch the objective symptoms, particularly the fever, cough and expectoration, noting whether they increase and again diminish or disappear within a day or two after the administration of a dose. Excepting in the upper air passages and other external surfaces, the presence of focal reactions is, as already stated, a matter of inference from the symptoms and signs which they produce. A reaction which causes but a slight local effect insufficient in degree to give rise to definite manifestations, necessarily escapes our recognition.

A focal reaction may be very transient, or it may take a day or several days before it subsides entirely. If the accompanying fever is only slight and of short duration, the reaction may not be observed while the temperature is elevated. As a rule, we find it about twenty-four hours after the dose has been given; exceptionally we have observed it in so short a time as six or eight hours, and in one case after two hours; but we have also seen it delayed for thirty-six hours.

The same locality may react a number of times and, after failing to do so after a certain dose, a reaction may again appear in response to an increased dose. Other lesions, especially those in which marked percussion-changes suggest their being fibrocaseous or those which are the seat of a cavity surrounded by fibrous tissue, usually do not react to smaller doses, and often not until reactions have ceased and unmistakable improvement is present in most or in all other tuberculous portions of the lungs. In the course of specific treatment, we may find that the physical signs of the reacting locality improve or disappear entirely after one or several reactions, that a particular locality no longer reacts after increased doses to which others still respond, and that the same then takes place in other localities. Ultimately the reactions may be confined to a single locality, perhaps the seat of a cavity the secretion of which increases, showing many tubercle bacilli when previously there had been but few, or their appearance in the sputum when they had been absent in several prior examinations; râles may occur when the cavity had apparently been dry prior to the reaction. These and other manifestations of focal reactions have sometimes been misinterpreted, the observer inferring that the treatment has done harm, whereas in reality the reverse is true, the effect being one of stimulation which favors the exfoliation of caseous material and the healing of the lesion.[2]

Focal reactions occur in extrapulmonary tuberculous lesions the same as they do in the lungs. They are, however, more likely to be overlooked unless they are accompanied by decided symptoms. In our experience, the frequency of tuberculous affec-

[2] Observations of this sort have frequently been referred to us by physicians, some of whom appear to have interpreted the increase of tubercle bacilli in the sputum, in connection with a reaction, as a sign of danger and as a sufficient cause to abandon further specific treatment.

tions in other organs and parts, which develop in the course of the pulmonary disease, is greater than is commonly supposed. In our last report [3] from the Winyah Sanatorium we tabulated 1610 tuberculous affections in other organs and parts, which were observed in a total of 2760 cases of pulmonary tuberculosis, and afforded us many opportunities of observing focal reactions in other organs than the lungs. In not a few of these cases our attention was first directed to the extra-pulmonary affection by symptoms which appeared after the administration of the specific remedy, and the reaction thus aided us in their diagnosis.

Focal reactions upon visible surfaces are recognized more readily, and most clinicians are familiar with those which occur upon the skin and the visible mucosa of the mouth and pharynx. We believe, however, that they are often overlooked in the nose, nasopharynx and larynx, and that many times the presence of tuberculous lesions in these parts is not even suspected until the disease has made considerable progress, because often they are not examined unless decided symptoms have made their appearance.

This is probably the case still more often in tuberculous affections of other organs in which we have to depend entirely upon symptoms for evidences of reactions, especially when the latter are slight and when they disappear rapidly, as is usually the case. Inasmuch as we desire to avoid the occurrence of severe reactions and must guard against repeating or increasing the next dose before the reaction has completely subsided, it is important that we should be cognizant of all tuberculous localizations in a given case, and that any reaction which occurs should be recognized and considered in the further administration of the specific remedy. The upper air passages, and especially the larynx, are most frequently the seat of tuberculous changes, particularly after the pulmonary disease has reached the open stage, and we believe frequent laryngoscopic inspection and careful inquiry to be indispensable in respect to all symptoms which occur after each dose, especially when this has been increased. Slight lesions may be so situated that the hyperemia and swelling, which accompany the reaction, cause no local pressure or irritation, or the symptoms may not be sufficiently

[3] K. v Ruck and S. v. Ruck; *loc. cit.*, p. 18 (p. 139).

marked to attract attention. Other lesions equally slight, for instance, in the eye, upon the epiglottis or vocal cords, cause symptoms more readily.

Extrapulmonary tuberculous localizations may be present in the very early stage and may even precede those in the lungs. In children whom we vaccinated and in whose lungs no evidences of tuberculosis could be found, we observed focal reactions upon the mucous membranes of the eye, the nasal septum, the tonsils, and upon the skin; also in one case of a fistulous tract in the lower jaw, and often in enlarged lymph-glands. The focal reactions were sufficiently marked, so that we could not have remained in doubt even if we had not been confirmed clinically by the rapid disappearance and healing which followed in most cases without further treatment.

Focal reactions in tuberculous bones and joints may cause temporary pain and tenderness which, in the joints, is apt to be increased by motion; in superficial joints like the knee, ankle, elbow or wrist, they are often attended by more or less heat and swelling. Open ulcers in any part, and sinuses and fistulæ appear inflamed and swollen and their secretions become markedly increased. Intestinal ulcers often give rise to diarrhea during reactions, and, in the cecum and appendix, to spontaneous pain and tenderness on pressure. In the kidneys, pain on the side of the affected organs is often well marked as a dull aching, and the reaction may cause the presence of red blood-cells in the urine as well as an increase of tubercle bacilli, and in one case we have observed hematuria. Increased frequency of micturition is a common symptom of focal reactions in the urinary bladder; localized pain or aching, irritation, tenderness on pressure, and increase of secretion, alone or combined, are the symptoms which characterize focal reactions in any part of the body.

When such symptoms occur in organs and parts which are not accessible to direct examination, their repetition and subsidence after subsequent doses of the specific remedy constitute a guide to the further administration of the remedy, and may also be accepted as diagnostic.

Special caution is also required in the fortunately rare cases in which subjective or objective symptoms occur, after a dose of a specific remedy, which suggest a reaction in circumscribed

local tuberculous lesions in the central nervous system. According to our limited experience with such cases, their exclusion from specific treatment or the abandonment of the treatment, if symptoms occur which indicate a focal reaction, is not justified, although they enjoin caution in regard to doses and to their increase. Like other observers, for instance, Browning,[4] we have noted the disappearance of these symptoms and the recovery of the patient, under the continuation of specific treatment, in several instances in which the occurrence of focal reactions in response to the remedy could not be doubted, because the symptoms appeared and subsided repeatedly, especially on increasing the dose, until they finally disappeared entirely. We hold that these reactions are analogous to those which occur in other organs which are inaccessible to physical examination or inspection, and in which we also must depend upon objective symptoms for a guide in adjusting our doses.

In regard to the advantage or necessity of adjusting our doses to the occurrence of focal reactions, we realize that such a course is not always possible and that it is not followed by many physicians who employ specific remedies with satisfactory results. Being guided entirely by the temperature records of the patient, they conclude that no undue focal reaction has been caused if no fever follows or if there is only a slight rise in temperature. It must be conceded that this is probably true in a majority of instances, but it is likewise true that, if the temperature chart furnishes the guide for the increase of doses, focal reactions may be infrequent or may be prevented altogether, especially if, with the object of avoiding fever, the doses are kept small or are increased only occasionally and but slightly at any one time. Fever-reactions cannot be prevented with certainty if the doses are increased in a proper manner. We have, ourselves, observed no harm from them and have, indeed, witnessed the most remarkable local and general improvement after unexpected severe reactions which were accompanied by temperature rises of from two to five degrees Fahrenheit.

The fear of reactions dates back to the first introduction of old tuberculin, and any one who is familiar with the medical literature, or who had personal part in the experiences of that period, will remember that harm appeared to have followed

[4] Chas. C. Browning; Medical Record; 1914, LXXXVI, 325.

occasionally upon the large doses employed at first, in cases which could not be considered to have been unsuitable for specific treatment. In the chapter dealing with prophylactic vaccination, we referred to the conflicting views of other observers in regard to general and focal reactions, and we again remind the reader that beneficial effects of focal reactions were recognized by numerous observers at an early period after the introduction of tuberculin; they were first observed in lupus by v. Bergmann, and soon thereafter by others, especially in the prompt healing of open tuberculous ulcers and sinuses after a decided focal reaction had occurred.

Although we have, so far, seen no harm, and, as a rule, only beneficial results following upon reactions even if they were marked, we still guard against severe reactions, especially at the beginning of the treatment and in cases where our preliminary investigations or subsequent observations suggest caution, either because we find extensive disease, particularly evidence of much recent tubercle-formation, or because, the patient being delicate, weak or much under weight, we do not wish to take the risk of causing a loss of appetite and of weight even temporarily, which is likely to supervene when the reaction is attended by marked and prolonged fever.

Upon the basis of our firm belief that moderate focal reactions not only are not harmful, but that they are valuable for the stimulation of retrogressive changes and of healing in tuberculous lesions, and that excessive or continued focal reactions can usually be guarded against by careful observations, we increase our doses more or less rapidly, or we repeat them as long as a focal response occurs. When no further response to a given dose is observed in the tuberculous focus, the fact is confirmed by the absence of the local reaction at the site of the subcutaneous injection.

In very early cases in which the tubercles have not reached large conglomerate forms and have not yet developed into fibrocaseous infiltrations or discrete caseous or fibrocaseous foci the accumulation of toxic products within the lesions cannot occur as readily as in more advanced lesions. The tissues permit a better and freer exchange of lymph and blood, directly or by osmosis, and are thus subjected to the action of the free am-

boceptors of the body-fluids in a degree which may suffice to cause the tubercles to become retrogressive or to disappear entirely without focal reactions having occurred which were sufficiently marked to be recognized.

According to our observations, the degree of the reactions, which follow the administration of the body-extractives of the tubercle bacillus, appears to be influenced by the age of the preparation, and probably also by its mode of manufacture, and by subsequent exposure to varying degrees of temperature. The fresher the preparation is, the more power does it seem to possess to cause reactions. This power is preserved longer if the preparation is kept cool than if it is kept at room-temperature. We may explain this, as does Ehrlich, by the spontaneous conversion of the toxin into a less poisonous or a non-poisonous "toxoid." Ehrlich observed that this conversion takes place more readily under the influence of warmth and of age, but also that the production of antitoxin to the administration of the toxoid form of diphtheria toxin occurs the same as it does in the case of the unchanged toxin. This would conform with our observation that a maximal degree of general immunity develops regardless of the occurrence of marked reactions.

When our vaccine is kept at room temperature for several months, it shows a distinct loss in its power to cause reactions, although that of stimulating the formation of specific antibodies remains unimpaired; the same is true, in a lesser degree, for the keeping qualities of our Watery Extract. If kept in a refrigerator, both preparations retain their reactive power undiminished for a year and probably for a longer period. The changes that are responsible for this loss of reactive power may be due to a simple hydrolysis; in our estimation they occur in consequence of an autolytic action, because it is not possible that the necessarily present specific ferments of the bacterial cells have been destroyed by heat or chemicals under the precautions which we employ in their preparation. We believe this view to be supported by the common observation that preparations, in the manufacture of which heat is employed, show no such changes regardless of the temperature at which they are kept. A specimen of Koch's old tuberculin, which was made in 1891 and kept by us for twenty years at ordinary room-temperature,

still caused general reactions in the same doses that were effective at first, and the same was true in the case of certain extractives of tubercle bacilli, obtained by us ten years before by prolonged boiling, which had not been kept at a refrigerator-temperature.

Finally we find that the ferments, which are concerned, have an intracellular origin, because no change could be observed by us in tuberculin which we made by concentration *in vacuo* at a low temperature, or in Bouillon Filtré which represents the unheated bouillon upon which tubercle bacilli have been grown, and in which the presence of intracellular ferments of tubercle bacilli could not be expected.

The reactions which we have described were produced with preparations obtained from tubercle bacilli in their comparatively fresh state.

The subject of specific reactions in infectious diseases is of such great importance for an understanding of their etiology, diagnosis and specific treatment that a further discussion of their origin, significance and influence appears justified, with special reference to bacteriolytic immunity in tuberculosis, although much of that which is relevant has been considered in the present and in other chapters.

SPECIFIC REACTIONS IN TUBERCULOSIS IN THE LIGHT OF BACTERIOLYTIC IMMUNITY

The first parenteral introduction into the organism of foreign proteins and of certain lipoids is borne in comparatively large amount without producing manifest local or general symptoms. These foreign substances seem to awaken and to stimulate a latent function which is designed for the protection of the organism against the injected foreign substance; through it the organism can dispose of this substance more rapidly and completely when the introduction is repeated. The function requires a variable number of days to develop and, when it has reached its maximum, the organism can split and reduce a certain amount of the foreign organic substance promptly and completely. When the injected amount exceeds the limit, the reduction is not complete and then takes place gradually and, step by step, the injected material is subjected to cleavage in

the blood and tissues, resulting in intermediary, so-called *split-products* which are more or less poisonous and irritant in nature, and which cause local and general phenomena, designated as *reaction*. The severity of the reaction appears to vary with the nature and source of the foreign organic substance which is commonly spoken of as *antigen*.

When this antigen is blood serum from another species of animal, the reaction to the second parenteral administration may be very severe and may cause the immediate death of small experiment animals, by what is called *anaphylaxis*. Anaphylactic symptoms have long been known in the human subject and have been observed in various degrees after the repeated administration of antitoxic sera. Similar but less severe symptoms and signs of reaction are observed after the injection of proteins and lipoids from other sources, notably from bacteria.

The reactions under consideration are strictly specific, and it is necessary for their occurrence on a subsequent parenteral introduction that the foreign substance or antigen be identical in its molecular and atomic constitution with that which was used for the stimulation of the function responsible for the occurrence of cleavage. The tuberculous nature of an existing infection can be established by the administration of sufficiently large doses of a bacterial antigen. If this is identical, the injection will cause a reaction; if it is not identical, no reaction will follow, because, in that case, the injection is in fact a first parenteral introduction of the antigen and no clinically manifest reaction occurs to it.

The same laws appear to govern the absorption of bacterial proteins and lipoids in the animal organism and the whole theory of bacteriolytic and bactericidal immunity is based upon them. The positive result of a tuberculin test represents a specific identification of a bacterial protein. For the reaction to occur, the organism must have acquired the function of splitting and reducing the particular proteid substance which is contained in the test fluid. A positive reaction does not, however, indicate that the function extends to all proteins or lipoids of the bodies of the bacilli, or to all products of their metabolism or secretion. nor does it signify that all of them were present in the test-fluid. A reaction can occur as long as an identical relation exists with one of them. If the function has become active and

efficient for others which are not present in the test-fluid, or if the latter contains substances for which the function is only latent and therefore not manifestly effective, no reaction will occur to their introduction because, in one case, the substance which could be split or reduced is not present, and in the other case the function is only latent or is not developed sufficiently to cause the reaction.

In order to make sure that the organism has the power to split and reduce all toxic products that can be derived from tubercle bacilli, it is necessary to make separate tests with the individual toxic products, which must be isolated upon a biological rather than a chemical basis for the sake of exactness. Our studies with tuberculin and with the several proteins and lipoids, as we have been able to isolate them chemically, have shown, however, that the tuberculous organism can react to one of them and fail to react to the others. Our biological studies have confirmed the correctness of our view that the spontaneous acquirement of bacteriolytic immunity against the tubercle bacillus and its several constituents is accomplished slowly and gradually, and that it is frequently only partial and usually of a low degree; our experiments upon animals which we treated with fractions of proteins and lipoids, as they are present in our vaccine, appear to show that, in order to produce resistance to infection, bacteriolytic amboceptors must have been formed against all body-constituents of the tubercle bacillus.

Although our knowledge of the chemistry of the tubercle bacillus is still incomplete, the results of our own studies and of those of others have proved that its proteid and lipoid constituents are numerous and of highly complex constitution. They differ broadly in that some of them are products of living bacilli and that others are body-substances which may be obtained after their death. For extraction *in vitro,* some of the latter substances appear to require solvents which are not naturally present in the animal and human organisms, while others are soluble more or less readily in water and in normal salt solution; still others require an acid or an alkaline medium.

While we are not unmindful of the fact that the solubility or disintegration of tubercle bacilli *in vitro* and in the living organism need not be identical processes, and that the organism may have other resources to prepare the bacilli for absorp-

tion, it is to be remembered that these means are not present normally, but that they must be acquired specifically.

In applying the accepted theories of bacteriolytic immunity to the study of specific reactions of the tuberculous organism in response to the injection of specific products of the tubercle bacillus, it appears that the tissue-cells, which possess suitable receptors for them, have a wide and general distribution in the body. This is shown by the localization of tuberculous processes, which may occur in any organ but is particularly frequent in connective tissue. It is also the connective tissue in which local reactions are induced most easily, and our observations have caused us to conclude that specific amboceptors against the products of the tubercle bacillus originate in a large measure from the cells of this tissue. These studies also demonstrated that a local immunity can develop and that it may increase over and above the general immunity which has been acquired by the blood or by other normal or tuberculous tissues.

It is a general observation in tuberculous subjects that local, general or focal reactions to a certain dose of tubercle bacillus-toxin grow less after successive injections, and cease entirely unless the doses are increased, and that in time these toxic products may fail to cause reactions even if the doses are doubled or increased still further. For local reactions, this stage is reached more quickly when several successive doses are injected in the same place; in this case we find that the same and often a much smaller dose will induce them in a new locality in which no previous injections have been made. It is therefore evident that something intervened which prevented the reaction in the non-reacting locality. Such observations used to be explained by the convenient term of *toleration;* in the light of specific bacteriolytic immunity, they mean that the injected toxic product is intercepted and bound by amboceptors which are specific for it, and that its local and general action is prevented by its prompt and complete reduction. In consequence the beneficial effect of the dose or of several doses may be lost. This does not occur in the new locality, because there the local immunity has not reached the same degree.

Like observations may be made by repeating the experiment in other localities which have not been used for subcutaneous injections of the specific toxic substance; a gradual increase of

the local immunity will be found to develop in new localities, and in time larger doses will be required to produce a reaction at any new site.

The theory which assumes an overstimulation at the site of repeated injections has not escaped our consideration in attempting to explain clinical observations; but it had to be abandoned when it could be shown that the reactive power of the cells, to produce specific amboceptors, was not exhausted or paralyzed, since local, general and focal reactions appeared again when the doses were increased sufficiently. Our experiences in this respect are so numerous and were accumulated in the course of so many years of observation that we cannot doubt the truth of the facts as they are stated, and they can easily be verified. These observations can also serve to explain the occurrence of unexpected severe reactions in case an injection is made at a new site, and of failures in obtaining satisfactory clinical results under specific treatment, as well as in attempts of experimental immunization in animals.

Our explanation, that the toxic substance is actually bound and reduced to its simple elementary constituents, is supported by our uniform experience that febrile or focal reactions never occur to the subcutaneous administration of our vaccine in cases in which there is an entire absence of local reaction, whereas they may be observed when a smaller dose is injected in a new locality, which then also reacts to the injection. It appears, therefore, that a local immunity may develop at the point where a specific product of tubercle bacilli is present in a more concentrated state, or in larger amount, or both. If this is the case, the same may occur in tuberculous lesions when the conditions are favorable, and it may account for the rapid healing which we have observed after injecting the specific remedy directly into the tuberculous lesion or in its periphery. The increase of the local immunity can also explain the beneficial effects of focal reactions, and the local development of immunity may stand in relation to the presence of healed or healing lesions, which are frequently found in autopsies of subjects that have died of other diseases. A study of the recorded findings shows that, as a rule, such lesions are minute, indicating that the local immunity must have developed at an early period, probably before the immunity became general. Such an occurrence may

be favored by a reduced virulence and a low degree of growth-energy of the tubercle bacilli, or by a latent specific resistance which may have been acquired by previous inhalations or ingestions of non-virulent bacilli which reached the lymph-glands, or by transmission *in utero*. The necessary condition is that the latent specific resistance becomes activated and that the body-substances of some of the bacilli, which entered the tissues, thereby become available to the local cells and enable them to produce and increase their specific bacteriolytic amboceptors.

A greater degree of immunity in local lesions may also account for spontaneous healing or for non-extension of the disease at later periods, and the insufficiency of the general immunity may explain the development of fresh tubercles at a distance, when none are found in the periphery of the local disease, or when the latter is evidently non-progressive or shows marked evidence of healing.

The results of our studies indicate that the mechanism of all specific reactions in tuberculous subjects is the same, and that all of them are conditional upon the presence of specific bacteriolytic amboceptors for one or all the toxic products of tubercle bacilli, and upon the presence of a greater amount of the particular toxic product than can be reduced to that elementary state in which it is no longer foreign to the tissue-cells and the blood. Unless these conditions are complied with, no reaction to the specific toxin can occur, either at the site of its introduction, or in the blood, or in tuberculous tissues.

Summing up the preceding remarks, it may be said that:

1. *Local reactions* occur in tuberculous subjects after the subcutaneous introduction of a sufficient amount of a toxic product of tubercle bacilli, because the tissues have spontaneously acquired a certain degree of immunity through the absorption of an identical toxin into the general circulation, which was followed by the development of specific amboceptors in the several tissues, the cells of which possess fitting receptors for the toxin. The local reaction depends upon the presence of the injected toxic substance in such an amount and concentration that the amboceptors in the tissues are inadequate to reduce it completely into its elementary simple constituents.

Since the disintegration of the toxic substance is then incom-

plete, intermediary or so-called split products of its cleavage accumulate and manifest their presence by a toxic action and irritation upon the local tissue-cells, which varies in degree and intensity, according to the amount and the degree of reduction which has been reached, and according to the derivation of the split products from one or the other of the bacillary toxic fractions. The size of the reacting area depends upon the local diffusion of the split products.

If no local reaction is produced by the injection of a certain dose of the specific toxic product, two explanations are possible. Either the identical toxin has previously not been absorbed in sufficient degree to cause the general development of specific amboceptors, or these were quantitatively and qualitatively ample to reduce the injected toxin completely into its elementary, non-irritating and non-toxic constituents. The validity of one or the other of these explanations will be shown by injecting an increased dose at a new site.

2. The occurrence of *general reactions* after the subcutaneous injection of a given toxic product of the tubercle bacillus depends upon the absorption of the latter into the blood, either in its unchanged or in a partially reduced state. If the reduction has not been fully completed by fitting amboceptors at the site of injection or in the blood, the intermediary split products cause toxic irritation of the nerve-centers, to which these respond with fever, general malaise and other symptoms. The degree of the general reaction depends upon the presence in the blood of split products in sufficient amount for a sufficiently long time; the reaction subsides with their disappearance by being bound or reduced further in normal and tuberculous tissues, or by elimination through natural channels.

If the available amboceptors in the local tissues are ample in quantity for the whole dose and for all its fractions, a complete disintegration takes place, and no split products reach the circulation. In the contrary case, more or less of the administered dose is absorbed and is then subject to reduction in the blood.

Inasmuch as the specific amboceptors of the blood are not likely to differ qualitatively from those which are present in the subcutaneous tissue, the reaction is similar, and split products, which were absorbed from the subcutaneous injection-site,

are not apt to be reduced further in the blood, although unchanged toxin may undergo cleavage.

3. *Focal reactions* depend upon the entrance of an amount of toxic substances into the circulation, which exceeds that which may be reduced completely in the blood or in the tuberculous lesions. When toxic products are present in the blood, they are in a state of great dilution and do not reach the individual tuberculous lesions in a concentrated form. Under favorable conditions, specific amboceptors may be present in large amounts in the local lesions; the smaller amount of toxin which is conveyed to them with the arterial blood may then be reduced completely by reason of the local immunity being quantitatively and perhaps also qualitatively greater than that pertaining to the blood. It is therefore possible that the general reaction is not accompanied by a focal reaction, although this is often the case. With the occurrence of a focal reaction, split products are absorbed from the reacting lesions, and these may intensify or prolong the general reaction, or may be solely responsible for its occurrence.

General and focal reactions may occur spontaneously under the same conditions which govern their appearance after the diagnostic or therapeutic administration of specific bacillary toxins. The general reaction then depends upon the absorption of unchanged toxin or of split products from tuberculous lesions which are in a state of reaction from incompletely reduced toxins. We believe that the catarrhal signs and the febrile phenomena in the early stage, as also those which occur in the course of phthisis, originate in this manner.

* * * * *

In connection with the subject of specific reactions, and their relation to split products, we must refer to the experiences of authors who could not produce the same local and general reactions in normal animals, after the injection of specific remedies, as they are observed in tuberculous animals or in tuberculous human subjects; yet such reactions must be expected to occur if our hypothesis is correct. We find indeed that local and febrile reactions can be produced in partly immunized, non-tuberculous animals, and that this is likewise the case in apparently non-tuberculous human subjects. We have succeeded more often in animals when we administered the toxin intravenously

and in large doses. The development of a local immunity at the site of subcutaneous injections has no doubt contributed to the failures which we and others have observed, inasmuch as in small animals, which receive numerous injections for treatment, most of the skin surface available for a cutaneous test has previously served for injections of the specific toxin, and positive results of such a test were then apt to occur only in exceptional cases. We have, however, observed reactions with sufficient frequency, in the course of the subcutaneous treatment of animals with vaccine, that we would expect more uniform success if we could devote the necessary time to the study of the subject. In the summer of 1913, we tried to take advantage of the common understanding that only actually tuberculous animals could react positively to a v. Pirquet test made with old tuberculin, because we had found so much spontaneous genuine tuberculosis in our animals that we wished to eliminate the affected animals by testing them cutaneously with tuberculin after the method of v. Pirquet. On killing and examining, with great care, the animals which had shown positive reactions, we found that some of them were absolutely free from genuine tuberculous disease, but also that this was the case only in animals which had previously been treated with vaccine; all other animals, which had reacted positively but had not been treated or infected by us, were found to have genuine tubercles in their organs. Our observations correspond therefore with those of Much & Leschke and of A. Jousset, who have observed positive cutaneous reactions in animals previously treated with products of the tubercle bacillus.

A number of animals, both treated and non-treated, which had not reacted to the cutaneous tuberculin test, were also killed, in order to determine the reliability of our observations. All were free from tuberculosis. The failure of some of the treated animals to react was then attributed to the development of a degree of local immunity sufficient to prevent the reactions, a view which could be substantiated further by the demonstration of specific amboceptors in their sera.

The subject of specific reactions is, we believe, of sufficient practical importance that an illustration of our own personal observation may be justifiable by the details from the clinical records of a typical case.

Mr. N. N.; age 26; admitted to the Winyah Sanatorium, October 5, 1913. Physical examination shows: a tuberculous affection in the right upper lobe, including a small secreting cavity, and slighter infiltration involving the left apex. Temperature 98.2° to 99.2° F.; moderate cough; expectoration about 10 cc. in 24 hours; usually early in the morning. T. B. found in sputum. Patient is in a fair state of nutrition and strength. Weight 138 lbs. Larynx shows infiltration of posterior wall. No other complications.

Oct. 12, 1913. First dose of vaccine, 0.2 cc., subcutaneously in left arm, caused marked local reaction. Temperature 101° F.; focal reaction in both lungs; negative in larynx. Expectoration 20–30 cc. on day of reaction, all signs of which disappear 48 hours after administration of the dose.

Oct. 17, 1913. Second dose, 0.2 cc. subcutaneously, same arm. Slight local redness and soreness at injection site; no focal reaction; no rise in temperature. Expectoration about 10 cubic centimeters.

Oct. 22, 1913. Third dose, 0.2 cc., in right arm. Marked local reaction; temperature 100.5° F.; focal reaction marked in left, slight in right lung; larynx negative. Expectoration 20 cc.; T. B. found.

Oct. 27, 1913. Fourth dose, 0.2 cc., in right arm. No reaction. Expectoration slight; could not be collected.

Oct. 30, 1913. Fifth dose, 0.4 cc., in left arm. Moderate local reaction; focal reaction in both lungs; larynx negative; temperature 100° F. Expectoration 20 cc.; T. B. found.

Nov. 4, 1913. Sixth dose, 0.4 cc., in left arm. No reaction; slight increase in expectoration.

Physical signs in left lung decidedly improved. Weight 142 lbs.

Nov. 9, 1913. Seventh dose, 0.2 cc., in right side of chest, anteriorly. Marked local reaction; focal reaction in right, slight in left lung; larynx negative. Temperature 100.2° F. Expectoration 25 cc.

Nov. 14, 1913. Eighth dose, 0.2 cc., in left side of chest. Slight local reaction; focal reaction negative in lungs and larynx. No change in expectoration, which is very scant; no T. B. found.

Nov. 19, 1913. Ninth dose, 0.4 cc., in right side of chest, posteriorly. Marked local reaction; marked focal reaction in right lung; negative in left lung; well-marked turgescence of infiltration in posterior wall of larynx, including both vocal processes; sense of irritation in larynx. Temperature 100° F.; cough increased; expectoration about 20 cc.; T. B. found.

Nov. 24, 1913. Tenth dose, 0.4 cc., in left side of chest, posteriorly. Slight local reaction; doubtful focal reaction in right lung; left lung and larynx negative. Temperature normal. Expectoration not enough to be collected.

Nov. 30, 1913. Eleventh dose, 0.5 cc., in right thigh. No reactions.

Examination of lungs: Right lung, cavity dry, area of physical signs greatly diminished; left lung, no signs; larynx infiltration appears less. No cough; no sputum. Weight 146 lbs.

Dec. 3, 1913. Twelfth dose, 0.8 cc., in upper part of abdomen. Marked

local reaction; focal reaction in right lung over cavity (râles on coughing); left lung negative. Larynx marked reaction; hacking cough; no expectoration. Temperature normal.

Dec. 8, 1913. Thirteenth dose, 1 cc., in left thigh. No reactions.

Dec. 12, 1913. Fourteenth dose, 1.5 cc., in right side between shoulder blades. No reactions; temperature normal; no cough; no expectoration; weight 151 pounds.

Physical examination: Right lung, marked retraction subclavicular over cavity, over which, and surrounding it, the percussion-note is short. Bronchial blowing is heard with expiration over the cavity; the inspiration is harsh over its periphery and lapses gradually into an exaggerated and normal vesicular quality in the second interspace. Posteriorly the findings are similar but less pronounced. The left lung appears normal. The larynx infiltration has disappeared. Patient is dismissed as apparently recovered, 68 days after admission.

Examination, *May 12, 1915,* 18 months after discharge: No change, except that retraction on right side is more marked; 1 cc. vaccine fails to cause reaction. General condition good; temperature normal; no cough; no expectoration. Weight 156 pounds.

In this connection another case, now under treatment, is also of interest. The examination of this patient shows a moderate degree of lung involvement, and also a tuberculous ulcer upon the mucosa of the left cheek and an erosion upon the left half of the lower lip. The ulcer is typical, and miliary tubercles can readily be seen in the edges. It was diagnosed and confirmed as tuberculous, by the physician who referred the patient to us, on histological examination of a piece of tissue excised from the ulcer. The patient had received four doses of vaccine, subcutaneously, of 0.1,—0.2,—0.3,—0.4 cc., to the first and third of which he had shown moderate general and marked focal reactions in the lung; but no reaction occurred in the ulcer, which was painful and annoying. In order to produce a curative focal reaction, 0.05 cc. of vaccine was injected locally into the edge of the ulcer. A severe focal reaction followed, causing marked swelling, heat and redness of the entire cheek, as well as of the ulcer and of the surrounding mucous membrane, which was accompanied by fever. The ulcer then became clean, it was nearly healed at the end of one week, and caused no further discomfort after the reaction had subsided. The erosion of the lip healed, although the lip did not participate in the focal reaction.

Seven days later the patient received a second slightly larger dose, which was injected locally, as before, and was followed by an equally severe reaction in which the left side of the lower lip participated, there being a sharp line of demarcation at the median line. The temperature reached 101.5° F.; but the fever and the focal reactions subsided more rapidly than on the first occasion.

With the subsidence of this reaction, granulations had filled the more deeply excavated parts of the ulcer, and the surface was covered over smoothly by the mucosa.

One case of tuberculosis of the knee joint was of interest on account of the coexistence of an old encrusted lupus on the corresponding leg, which had involved the greater part of the cutaneous surface, in addition to a moderate infiltration of one lung, which however had caused no symptoms. The knee joint was enlarged, swollen, and sufficiently painful on walking to oblige the patient to use crutches. The swelling showed little fluctuation and was more suggestive of massive granulations than of a fluid exudate.

This patient had received only three doses of vaccine when the joint appeared to be normal in all respects. The injections were made in the neighborhood of the diseased joint. The first two doses caused severe reactions attended by fever, increased swelling, heat and tenderness of the joint; during this time the patient was unable to use the limb without severe pain; the lung-lesions reacted only slightly, and no reaction was noted in the skin-lupus, although the crusts disappeared after several additional doses of vaccine had been given, and then the lung-lesions also disappeared. The patient, who has been under our constant observation since treatment, two years ago, has suffered no relapse and is in perfect health.

We have proceeded in the same manner in other tuberculous ulcers of accessible mucous membranes, making the injections in the neighborhood of the lesions, or by administering the vaccine intravenously and also in tuberculous affections of bones and joints, lymph-glands, peritoneum, genitalia, and in sinuses and fistulæ, always with rapid and satisfactory results whenever we were able to cause decided focal reactions.

THE VALUE OF SPECIFIC TREATMENT

We have stated repeatedly that cases presenting non-complicated, purely tuberculous processes constitute the really eligible ones for specific treatment. We see the confirmation of this assertion constantly in daily practice; it is evidenced by the permanency of the ultimate results in these cases, and also by the rapidity with which clinical results can be secured. We have shown likewise that, when the tuberculous changes are incipient or only slight, a single full dose may be effective and sufficient to cause the disappearance of all signs of tuberculous disease.

Only in this early stage is it possible to demonstrate the full benefits of specific treatment by the uniformity of the results; the fact that such cases often do well without it does not at all justify a skeptical attitude or furnish an excuse for first awaiting the outcome of dietetic, hygienic and climatic treat-

ment. The facts are that a considerable number of early-stage cases fail to make an apparent recovery, and that a still larger number relapse, even after many months of general treatment, and that then they must be treated again; but in the meanwhile they have passed into a more advanced stage which offers less prospect of success. In these cases nothing was really accomplished by the general treatment beyond a temporary general improvement and subsidence of symptoms.

The success of the treatment is all too frequently measured by the number of pounds which the patient may have gained in weight, instead of by the local improvement shown by the disappearance of physical signs, or by the retrogression of the disease, as it may be inferred from the character of those signs which still persist. The weight-gain is often particularly misleading in patients of the poorer and laboring classes, after they are brought under favorable conditions, as for instance, when they enter public sanatoria or hospitals. These patients have often been living under more or less unsanitary conditions, on a poor or insufficient diet, and have been working until the hour of their departure from home. The better food in the sanatorium is tempting to them; they eat more and with better relish; they are relieved, may be for the first time in years, of the necessity of stress and toil; they enjoy a vacation under wholesome guidance and advice which prevent excesses and injury, and in consequence the progress of the disease slackens or stops, and the symptoms improve in proportion. The weight-gain very naturally affords the most striking and manifest evidence of this improvement. After these patients are discharged from the sanatorium in which they may have spent three, four or more months, relapses are not necessarily to be expected the moment they return to their previous environment and to their former mode of life; on the contrary, the disease may now remain quiescent or even become retrogressive, provided exciting causes do not intervene through which the tuberculous process is rekindled. In other words, having experienced, before admission to the sanatorium, an acute exacerbation of their disease, which led them to seek medical advice, and having overcome it by several months of a suitable regimen under favorable conditions, the patients are now again where they were before their symptoms caused them to seek aid. The experience of the first decided

activity of the previously quiescent disease, and the instructions received in the sanatorium while under treatment for a time or permanently, may increase the cautions which are to be practised in order to prevent relapses, and it is possible that a lasting recovery may result. An attack of influenza, however, or an accidental exposure to cold, stress, strain, dissipation or deprivation, occurring before the recovery has advanced far enough or has been established definitely, may bring on a new relapse, and this the more surely the shorter the time is that nature has had in promoting the process of final healing, which is always slow when unaided by suitable treatment.

Such improvements, which may simulate cures, may occur again and again after more or less marked relapses, and it was in observations of this kind that the dictum "once tuberculous, always tuberculous" had its origin. Physicians who depend upon climatic influences recommend permanent residence in the favorable climate for the same reason, in order to eliminate unfavorable climatic factors which may be responsible for relapses.

Those who, like one of the writers, have treated pulmonary tuberculosis for years, before even a correct conception of its etiology had been obtained, and thereafter with various measures, in addition to the hygienic, dietetic and climatic methods, in the way of germicides and of other agents, chiefly chemical, and who have observed and followed their cases after discharge, realize that the results which can be obtained with the advantages of specific treatment were not possible of attainment at that time. Others, who still hesitate to consider the specific treatment which is etiologically correct, must realize that their results fall behind so greatly in the percentages of apparent recoveries and arrestments of tuberculous disease and in the permanency of results which follow on the addition of specific treatment, that it should cause them at least to give a fair trial to a method which accomplishes so much more, even in the hands of general practitioners who make no pretensions of being specially equipped or skilful in the treatment of tuberculosis, but who accept and manage the cases of this disease in the ordinary course of practise, just as they accept and treat all others. If, with the adoption of specific treatment in uncomplicated early-stage cases, the results are judged not only by the amelioration

of symptoms but also by the comparison of carefully recorded physical signs before, during and after the treatment, it will be realized that the specific treatment leads to prompt recovery in practically every case of that stage, without the mistaken special dietetic and hygienic regimen which consists in stuffing the patients with milk and eggs, or in exposing them to wind and weather, upon the fallacious theory that milk and eggs have some mysterious therapeutic or nutritive virtues which accrue more particularly to tuberculous persons, and that tuberculous individuals are peculiar in needing to live out of doors by day and by night, in order to improve and get well. Extremes are applicable in medicine as little as elsewhere, and therapeutic methods that have degenerated into fads have thereby lost their usefulness, because this fact prevents the necessary individualization in applying methods which were originally designed to assure, for the tuberculous patient, the best possible nutrition and a constant and complete ventilation of his respiratory organs.

In cases that have advanced lesions, the value of specific treatment lies, in our opinion, chiefly in the completion or increase of an existing specific immunity or resistance to the tubercle bacillus, and thereafter in focal stimulation, by which an increase of local immunity and the healing of the tuberculous processes may be secured. As to the former, we find that a considerable number of patients have acquired, spontaneously, a sufficient degree to hold the disease in check; at all events, specific antibodies are found more frequently in the sera of patients whose physical examination gives evidence of destructive changes than is the case in the early stage. Further, their sera show a greater bactericidal power *in vitro,* as is evident from the greater duration of life which we noted in the autopsy-records tabulated on pp. 366 and 367, of animals infected with tubercle bacilli exposed to the action of serum taken from the more advanced class of patients, before specific treatment was employed. In the light of the pathology of tubercle and of the clinical course of tuberculous disease, this is to be expected; if the specific resistance also can be transmitted, or can be acquired during fetal life, as our studies of sera taken from infants at birth and at various periods thereafter appear to indicate, it is still easier to understand the variability of the clinical course of tubercu-

losis, and to appreciate why, in some instances, exposure is not followed by infection at all, why in others the infection is not followed by clinical disease, and finally why in still others more or less prompt improvement, arrestment and even recovery occurs after caseous softening and excavation of a small lesion have taken place. Neither is it then astonishing if the observation is made that highly virulent tubercle bacilli in cavernous sputum, or even in the blood, so rarely cause local or generalized tubercle, and that individuals who harbor them may live for years in a comparatively fair state of health.

For an explanation of all this variable behavior, it is of course necessary to formulate theories, and these theories must be based upon the evidence which is available at the time. While we appreciate that our own theories may be erroneous in part or wholly, they nevertheless give encouragement for further studies until it may be possible to separate the wheat from the chaff. As far as we have gone at this time, we are working upon the theory that the tubercle bacillus is an absolutely foreign body to the mammalian organism; that this has originally neither an acquired nor a hereditarily transmitted specific resistance, and that in such an organism the bacillus finds optimal conditions for its growth and multiplication, providing it has previously been accustomed and adapted to the nutrient soil which the organism supplies. In the human subject, the tubercle bacillus, accustomed and adapted to its soil, would therefore manifest a higher degree of virulence and of growth-energy than it would if it had grown in the tissues of another species of mammalia, this is in accordance with the facts elicited by the evidence at hand.

If living tubercle bacilli, accustomed to the soil of the human organism, are not modified in their growth-energy by EXTERNAL conditions to which they may have been exposed, after discharge from the lesions of their prior host (temperature, sunlight, desiccation, putrefaction, etc.) or by INTERNAL conditions to which they have been exposed before being discharged (prolonged retention in non-vascular, caseous, fibrous or necrotic foci; bactericidal action of the solid and fluid tissues of the prior host), they possess the maximal degree of virulence for humans; if they enter the tissues of an individual who possesses no transmitted or acquired specific resistance, they grow and multiply with

maximal rapidity and cause acute generalized tuberculosis. Such a non-resistant organism then becomes overwhelmed; the bacilli do not remain localized, neither do they degenerate; the formation and dissemination of tubercles is rapid, and caseation is not a marked feature. There is no complete immunizing response on the part of the invaded organism, except to the specific toxic products which the tubercle bacilli elaborate in the course of their own evolution, and these are formed and absorbed so greatly in excess of the power of response that the corresponding amboceptors and lytic ferments are quantitatively inadequate to bind and neutralize them. Instead of protecting the organism from harm, they cause toxemia due to the absorption of intermediary split products, and these are responsible for the final exhaustion and death which follow inevitably.

Many cases of this kind are on record in literature, and almost all experienced physicians have observed them. They are more common to peoples and races which, like some Indian tribes and other primitive peoples living remote from contact with civilization, have previously not come in touch with tuberculosis.

Bearing in mind the external and internal conditions referred to, which can modify the virulence of the tubercle bacillus, it is easy to understand why a like evolution of disease need not occur in every individual, even though a specific resistance may be lacking entirely; and further, why the period of evolution may vary according to the number of tubercle bacilli which find access, or according to the reduction which their virulence has suffered. It is self-evident that it may require one or two generations of the bacillus, or even more, before a maximal growth-energy is restored and, during this time, local tissue-reactions and a local immunizing effect may cause at least a temporary, if not a more lasting, check to generalization.

In instances in which the individual possesses a transmitted specific resistance, the evolution of an infection may fail entirely because this resistance is sufficient to prevent growth and multiplication of the tubercle bacilli, especially when they are not fully or maximally virulent, and in this case it may come to a latent infection or to the presence of tubercle bacilli in the tissues, especially in the lymph glands, without tubercle-formation. If tubercles are actually formed, they may be limited in number, because of the local tissue-reaction, and may become

converted into fibrous tissue; again, caseation may occur and the tubercle bacilli may then become encapsulated, as is evident from the healed and obsolete lesions found on autopsy of clinically non-tuberculous individuals. Like results may follow when the infecting virus is weak in virulence, as has been explained, in those individuals who lack a transmitted specific resistance of sufficient degree; or then both factors may cooperate in checking the further evolution of the tuberculous focus.

Once the lesions have become caseous, they are a menace to their possessor for many years; in fact, until they are entirely obsolete. During all this time tubercle bacilli may escape and reach the periphery or even distant parts, leading to a repetition of tubercle-formation and to like reactions, and eventually to large fibrocaseous nodes or to multiple lesions of varying sizes and of similar character. Such a course depends upon one or both of two factors, the one being an inherited or acquired specific resistance, the other a reduction in the virulence of the virus; both are subject to quantitative variations. When the virulence of the virus is the only factor, complete healing may fail, because this virulence may increase during successive generations of the bacilli. In such cases healing depends on the simultaneous occurrence of local cellular and of humoral immunity in a sufficient degree to keep down the virulence or to suppress it entirely.

We have no doubt that a slight degree of immunity to one or more of the toxic products of the tubercle bacillus develops in all cases, and the fact that it is not possible to demonstrate specific amboceptors in the blood-serum of a given case does not disprove this, because the complement-fixation reaction is too coarse for such a purpose. We find specific agglutinins for the tubercle bacillus in practically all sera of early cases, and this suggests an immunity reaction. This view finds support if the germicidal action of the sera of infected children, taken before vaccination, is compared with that of the sera of normal guinea-pigs or rabbits, and it is supported clinically by the occurrence of so-called latent or apparently non-progressive cases.

The natural, spontaneous acquirement or increase of germicidal immunity against the bacillus of tuberculosis must necessarily be slow, incomplete, and quantitatively slight during the

early stage of the disease. A marked increase of this spontaneous immunity cannot be expected until, with the absorption of tubercle bacilli, or of their endotoxins from destructive lesions, the organism becomes capable of making an effective response by producing specific amboceptors for all bacillary substances.

The spontaneous active immunization of the organism against the tubercle bacillus itself commences with the absorption of the liquefied caseous material, in the sense that endotoxins of the tubercle bacillus then reach the blood in sufficient quantities to stimulate the cells of the organism that they can produce the required specific bacteriolytic amboceptors in maximal amounts. Unfortunately, however, the disease has often made great headway at this period, and the destructive processes involve larger foci, in consequence of which the absorption is often so excessive that rest alone is insufficient to reduce it within safe limits. The intake of these specific products is necessarily governed by the size and number of the lesions and by their richness in tubercle bacillus products, by the rapidity of softening and by the condensation, or otherwise, of the tissue that is peripheral to the lesion. Ordinarily the absorption is at first slow, irregular and intermittent, and the symptoms are correspondingly so. Slight elevations of temperature, malaise, loss of appetite, appear and disappear, and often they seem to stand in relation to exercise and rest, so that the regulation of both has been taken advantage of in bringing about the desired auto-immunization. More absorption being caused by exercise, this corresponds to the administration of a dose of the specific toxin, while absolute rest affords the opportunity for the reaction to subside. There can be no doubt that certain patients can acquire their immunity in this manner without artificial specific treatment, and the fact is attested by clinical observations which antedate our knowledge of immunity, as for instance the clinical benefits accruing from exercise or from rest, for which a commensurate explanation is now possible.

In certain torpid, chronic, non-febrile cases, exercise stimulates the production and absorption of immunizing substances from the tuberculous focus; if this absorption is not excessive, a beneficial result is observed to follow. It is probable that observations of this kind led Sydenham to advocate horseback riding for consumptives, and caused Benjamin Rush to pre-

scribe a carefully graded plan of exercise, commencing with passive motion in the swing and ending in horseback exercise; these observations also account for the custom prevailing, not many years ago, to send consumptive patients out West to "rough it." On the other hand, cases in which tubercle bacillus products are already being absorbed do better under rest, in that the absorption and, with it, the fever diminish, and improvement often follows in the clinical signs and symptoms of the disease. This improvement was naturally attributed to the rest; in reality it should be credited to the prevention of an excessive absorption of bacillary substances through which the specific antibodies, which were being used up for the reduction of the specific toxins, became available for their action upon the tuberculous lesions. It is therefore easy to understand that clinical observers could adopt opposing attitudes toward exercise and rest in the management of consumptive patients, and why both methods have been followed by good results.

By applying these methods now with a definite aim and purpose, as was done first by Dr. Paterson, of Frimley Sanatorium, the results have become better in proportion as the absorption of toxic products can be regulated in conformity with the requirements of the individual case; but this method of promoting the process of auto-immunization in tuberculous disease appears to us to be comparatively crude. The good results which have been observed only support the value of deliberate artificial immunization and of specific stimulation under conditions of better control.

In the case of larger lesions, in which softening and absorption occur rapidly, the signs and symptoms increase in proportion, and their subsidence depends more particularly upon the time when the pent-up contents of the tuberculous abscess will gain a free exit. The desired relief may come as early as within a few weeks, but it may also be delayed for a number of months, during which the attending symptoms may vary in the widest limits; they do not subside permanently until complete drainage is established. Often this fortunate termination is not realized because other caseous lesions undergo like changes, resulting in coalescing and multiple excavations during which the patient becomes exhausted, or death is caused by intercurrent pneumonias due to the aspiration of cavity-secretions. Dur-

ing this entire period the patient has been in a more or less continuous state of general and focal reaction, and his mechanism for the production of specific antibodies may have been overstimulated so greatly that, when the absorption of toxic products finally subsides, it fails to respond, being practically paralyzed. Such a lack of response we believe to correspond with the disappearance of fever, as it is observed sometimes after a long and tedious course of excavation and suppuration, for longer or shorter periods, before the disease terminates in death from exhaustion, with an extreme degree of emaciation.

On the other hand, we have witnessed the advent of softening, and the formation, complete drainage and cleaning out of a cavity of moderate size, in the course of a single month, with only slight focal and general reactions, the only evidence being the recurrence or increase of bacillary expectoration. Such a course has occurred, we believe, almost exclusively in patients who had previously received specific treatment and who were completely immunized before the necrotic material softened and was absorbed.

In regard to patients who have passed the early stage as well as that of softening, and who are left with open secreting cavities, with slight or no fever, our observation is that their sera, as a rule, do not show a maximal degree of complement-fixation, and that they appear to have but a very slight power to cause morphological changes upon tubercle bacilli in bacteriolytic tests. The sera are rarely fully bactericidal, although the patients have passed through a longer or shorter period of softening and absorption of specific bacillary products. Such patients often react generally and focally to the administration of the specific remedy, and we have often been able to increase and complete the immunity and to bring about the desired further improvement by specific treatment.

In certain phases of the advanced stages, specific treatment can therefore aid our efforts toward securing an arrestment of the disease, and the only reason for withholding it in a far-advanced class of cases that have little or no fever, would be that the damage already done, or the complications present, or both are so formidable that no reasonable hope exists for clinical improvement and benefit.

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The GENERAL MANAGEMENT of patients under specific treatment is the same as it would be without it. Hygienic and dietetic methods with a view of securing the best physical condition of the patients, and of conserving and improving their nutrition when necessary, require unremitting attention and supervision. Complications should be prevented, rather than cured after they have occurred; if they are non-tuberculous in their nature, they should be treated upon the principles which apply in non-tuberculous patients, with this difference that prompt treatment is the more necessary in tuberculous patients, and of the greater importance, in view of their unfavorable influence upon the course of the tuberculous disease. We have often been asked for our opinion concerning the possible interference of other therapeutic measures, especially the administration of drugs, with the action of specific remedies, and we may say here that such an interference need not be apprehended; yet we avoid drugs, especially hypnotic and analgesic remedies whenever possible, if other remedial measures can be substituted for them. Physical comfort and suitable diversion, properly heated and ventilated apartments with modern sanitary appliances, the regulation of exercise and rest with reference to their influence upon the temperature and the heart-action, proper ventilation of the lungs by securing the opportunity of breathing pure, fresh air, the benefits of sunlight, the adjustment of the dietary to existing conditions, the maintenance of proper elimination through the skin, lungs, kidneys and bowels, proper hours for sleep, and the consistent cooperation of the patient,—none of these things have lost in importance by the fact that the patient receives specific treatment, and the same remains true in regard to benefits that may be derived from an advantageous change of climate, when the one in which the patient lives is rigorous and changeable or enervating, if it exposes him to malaria or to the acquirement of catarrhal affections, and if it prevents a sufficient exposure to sunlight and an adequate amount of exercise in the open air, to all of which the vegetative processes of the living organism react in a favorable manner. A change in locality, even if the one to be selected is not superior climatically, to the open country where the accumulation of dust and dirt in the air is at its minimum, or to a place where the change removes the patient from social and business affairs and worries,

and thereby diminishes and eliminates obstacles to the essential compliance with the indispensable cooperation on his part, is still as advantageous as it has been found in times past.

For the practical support of the principles and theories which govern us in the specific and associated methods of treating tuberculous patients, we refer to the clinical reports of the Winyah Sanatorium, especially to the one issued recently, which gives our results for the four years prior to January, 1915, and includes a résumé of the results in all cases treated in the institution since its establishment in 1888. It also includes the clinical experiences and results of many other physicians who have applied our methods; when added to our own, the experiences and observations reported represent a clinical material of over 5000 cases of pulmonary tuberculosis, and of numerous complications. We consider these statistical data to be of particular value because of the large number of cases treated with the same preparations, and because we are not aware that an equally large number of cases has been presented heretofore, in a form that is available for comparison and study, in that they were treated by the same observers, under the same diagnostic and therapeutic methods and prognostic classification.

In closing these practical discussions, we can only point out once more that the entire subject of immunity is by no means settled definitely, and that all explanations designed for its elucidation are theories and hypotheses, based upon observed facts. While we do not expect that theoretical research or animal experimentation alone will be able to discover the solution of all the problems involved in these studies, we maintain that the results and conclusions of the laboratory investigator are important and valuable, although they will always remain subject to practical confirmation at the hands of the clinician. We believe, therefore, that the results of our experimental investigations should be of interest to the clinician, especially as they have been made side by side with, and as many of them were first suggested by, our clinical observations.

PART III

EXPERIMENTAL STUDIES IN THE IMMUNIZATION AGAINST TUBERCULOSIS

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CHAPTER X

CHEMICAL AND EXPERIMENTAL STUDIES OF THE TOXIC CONSTITUENTS OF THE TUBERCLE BACILLUS

Our chemical studies of the several toxic constituents of the tubercle bacillus have not yet reached a stage to make possible individual investigations in regard to their toxic and antigenic action, with sufficient accuracy to ensure the correctness of the observed results. We have described, in our report of 1912, the ordinary chemical characteristics of the several extractives which we obtained from tubercle bacilli and which are present in our vaccine. Quantitative determinations of washed, dried tubercle bacilli, made since then, show that the total protein-content amounts to a fraction over fifty per cent, and that the lipoid constituents vary in amounts, averaging about thirty per cent.

In our renewed attempts to separate the several proteins and lipoids, for the purpose of studying them individually and more accurately, it still appeared desirable to us to avoid procedures which are liable to injure them; but our success was not much greater than before. In these more recent studies we found a marked difference in the toxic action, especially of one protein which corresponds chemically with a peptone, and of a lipoid extracted with absolute alcohol, both of which killed apparently normal guinea-pigs after the first administration of a minute dose (0.1 mgr.), whereas other proteins and lipoids were tolerated in doses that were from fifty to one hundred times larger, without causing the slightest disturbance. It was then also found that no apparent harm followed if the first dose of these toxic products was reduced to one-tenth of the minimum fatal dose, and that thereafter the doses could be increased gradually without producing a toxic effect. Complement-fixation with the sera of animals which had received the peptone, gave negative results with it as antigen and with all other T. B. antigens. Ani-

mals which had been treated with several other protein-extractives or with their fractions, as a rule showed complete complement-fixation with the particular extractives or fractions, and slight degrees, or only traces of binding with others, frequently including the lipoids. In sera from animals treated with lipoids, especially with those extracted with alcohol, the fixation was frequently complete also with one or several proteid antigens, although none were presumably present in the lipoid used for treatment.

The only exception was observed in sera of animals which were treated with our Protein No. 1, in which fixation was complete with it only, to the exclusion of even traces with all other partial antigens. These sera reacted, however, positively also with old tuberculin, and animals treated with the latter often showed the same titer in complement-fixation with tuberculin and with this protein.

In comparative chemical examinations of the unconcentrated and unheated bouillon upon which tubercle bacilli had been grown, and of Protein No. 1, we observed identical reactions in that their solutions became turbid upon the addition of dilute hydrochloric and acetic acids; after acidulation with 10% HCl and heating slowly, turbidity appeared at about 42° C., and distinct coagulation at about 48° Centigrade. The Protein No. 1 assayed 5 per cent of heat-coagulable albumin of its total organic substance. Plain sterilized bouillon, used for controlling the reactions, gave only negative results. We further found that we could obtain our Protein No. 1 from the liquid media of very young cultures, when the growth was actively progressive and was spreading rapidly over the surface of the bouillon. After its removal, the bacillary mass falls apart readily and the bacilli seem to have lost the adhesive substance which binds them together.

Formerly we had believed this protein to be one of the body-constituents of the tubercle bacillus, but, judging from the results of experiments made with it some years ago, we had deemed it as of little if any value for active immunization, and it is on that account that only a small fraction of it is included in our vaccine. In the light of the observations made since then and described above, it has become very doubtful that this protein is one of the body-substances of the tubercle bacillus; it

appears to be a product of living tubercle bacilli, which they secrete and which adheres to them and then becomes dissolved in the liquid medium upon which they grow. We believe, therefore, that it is identical with a protein contained in tuberculin, and the latter contains also the heat-coagulable albumin when it is prepared without heat.

No correspondence of action could be observed in comparative studies of diagnostic reactions, to ten per cent solutions of old tuberculin, of unheated culture-medium from which the tubercle bacilli have been removed by filtration, of Bouillon Filtré, and of our Protein No. 1. Although all these products were capable of causing reactions by the v. Pirquet method, these appeared most constantly after the injection of old tuberculin; they were less frequent with the unheated culture-medium and Bouillon Filtré, and rarely so with our Protein No. 1, in tuberculous individuals upon whom the tests were applied simultaneously.

If we explain these differences by the degree of specific immunity which the tuberculous subject may have acquired to the several toxic products of the tubercle bacillus, we must necessarily conclude that this degree was higher against some of the toxins employed than against others, or that no immunity was active at all against those to which no reaction occurred. In order to determine this point, the several preparations were then used subcutaneously, with similar results. The largest doses were required of our Protein No. 1, smaller doses sufficed of the unheated culture-fluid and of Bouillon Filtré, and still smaller doses caused reactions to old tuberculin. We conclude from these results that tuberculous patients are apt to possess a higher degree of immunity against our Protein No. 1, and least against old tuberculin, and in consequence we are justified in assuming that the several toxic products differ in that some are present in tuberculin which are not contained in unheated culture-medium and in Bouillon Filtré, and that some are present in the former which are absent in our Protein No. 1. Observations of their toxic action upon tuberculous guinea-pigs confirmed us further in this view.

These observations suggest the presence of an additional specific component, or of several, in tuberculin, whether this be prepared with heat or not, which differ from our Protein No. 1;

they caused us to reconsider certain studies made in our laboratory twenty years ago. It will be recollected that objections were raised, soon after the introduction of tuberculin, against its clinical use, because of the presence in it of tubercle bacilli and of acid-fast fragments. In order to eliminate this objection, a tuberculin was prepared in our laboratory which was freed from tubercle bacilli and acid-fast fragments, by first passing the culture-medium, upon which they had been grown, through Pukal or Berkefeld filters, before concentrating it to one-tenth of its volume. It was, however, soon found that tuberculin prepared in this manner was not reliable in producing reactions, and it was also less toxic for tuberculous guinea-pigs. This specially filtered tuberculin was inferior also therapeutically, and the conclusion was unavoidable that, by filtration, we had removed a part of the active principle which, under the circumstances, we believed to have been of corpuscular nature, consisting of bacillary bodies or of fragments and granules. We were, therefore, obliged to revert to the former method of concentration and to repeated filtration through hard paper or other less perfect filters, in order to obtain the desired uniformity of reactions in tuberculous subjects for diagnostic purposes.

These studies were then extended to an inquiry into the relation of the age of the cultures used for the manufacture of tuberculin, and it became evident that old degenerated cultures yielded a better therapeutic preparation.

We took advantage of this in the manufacture of a Purified Tuberculin, by resorting to the use of cultures upon which all growth had ceased, the bacillary mass having broken up and settled on the bottom of the flask. On observing that passage of the finished preparation through Berkefeld filters had apparently not diminished its specific action, we concluded that certain valuable substances were extracted from the bodies of the tubercle bacilli by their prolonged maceration in the culture-medium, a view which was supported by the better clinical results which we and others observed under its therapeutic administration, and which induced us to adopt a still more effective method of extracting the bacilli by boiling the morphologically degenerated cultures *in vacuo* at 45° C. for a month or six weeks, before we filtered out the bacillary residue.

This was the first deliberate attempt made by any one, to

extract and to incorporate the soluble body-substances of the tubercle bacillus in a preparation, and to employ it clinically for the specific treatment of tuberculosis. The clinical results obtained with it were so satisfactory that we eliminated the culture-medium entirely and depended upon the extractives alone, which we introduced in the spring of 1897, in the form of the Watery Extract of Tubercle Bacilli.

A renewed chemical examination of the Watery Extract was undertaken in 1912, because we had found that, after its administration, complement-fixation tests were positive in maximal degrees with all antigens, including the lipoids and old tuberculin; and it was because of these apparently contradictory findings that we questioned the necessity of the lipoids in the vaccine, in our first report on prophylactic vaccination. Events have shown that very small amounts of the lipoids are contained in the Watery Extract (Protein No. 2), and that it contains also Proteins Nos. 1 and 3 in larger quantities. The presence of Protein No. 4, in the Watery Extract, was found to depend on the reaction of the solution; the protein is present when the reaction is amphoteric, and it is precipitated when the reaction is made neutral or faintly acid.

The positive and complete results in complement-fixation, with sera tested after vaccination or after treatment with Watery Extract of Tubercle Bacilli (Protein-Extractive No. 2) having been explained by the presence of more or less of the other body-constituents of the tubercle bacillus in it, we made the attempt to free it at least of the lipoids, so far as this might be done, by shaking them out with chloroform and with sulphuric ether, in order to determine whether these substances were necessary for the protection of animals.

The sera of animals treated with Watery Extract before and after the removal of the fats gave only slightly different results. The outcome of the infections in the treated animals unfortunately was obscured by the occurrence of early deaths on account of pseudo-tuberculosis. No positive conclusions could be drawn, because only one of the animals lived longer than thirty days after infection.

In our serological studies, after treatment with the lipoids as prepared by Much, and with lipoids extracted with ether and with absolute alcohol without previous saponification, we

COMPARATIVE RESULTS FROM IMMUNIZATION OF GUINEA-PIGS WITH PARTIAL ANTIGENS AND OTHER PREPARATIONS DERIVED FROM TUBERCLE BACILLI

All infections were made subcutaneously with 0.01 mgr. of virulent tubercle bacilli of human type

Preparation Used for Treatment	Number of Doses, Average	Total Milligrams, Average	Number of Animals Treated or Used	Died Before 30th Day After Infection	Number of Animals Considered	Had Tuberculosis				Free from Tuberculosis				Complement-fixation					Bacteriolysis	Remarks
						Genuine Only		Genuine & Pseudo		Died		Killed								
						Animals	Average Days of Life	Animals	Average Days of Life	Animals	Average Days of Life	Animals	Average Days of Life	Preparation Used for Treatm	T.B. Emulsion	Neutr. Fats	Fatty Acids	Proteins		
Vaccine, Whole.....	11	56	8	1	7	—	—	—	—	4[1]	70	3	260	+	+	+	+	+	++++	[1] Four animals died; had pseudo-tuberculosis
Protein No. 1, whole...	15	75	8	5	3	1	79	2	44	—	—	—	—	+	0	—	—	0	0	
Prim. proteose.....	15	75	8	4	4	3	123	1	46	—	—	—	—	+	0	—	—	0	0	
Second proteose....	15	75	8	5	3	3	98	—	—	—	—	—	—	+	0	—	—	—	0	
Heat-coag. protein..	15	75	8	6	2	1	80	1	46	—	—	—	—	+	0	—	—	tr.	0	
Peptone.....	15	32	8	5	3	3	62	—	—	—	—	—	—	tr.	—	—	—	—	0	
Protein No. 2 (Watery Extract).....	12	93	12	5	7	—	—	1	115	—	—	6	202	+	+	+	+	+	marked	
Prim. proteose.....	18	59	8	6	2	2	158	—	—	—	—	—	—	+	tr.	—	—	tr.	0	
Second proteose....	18	59	8	7	1	1	117	—	—	—	—	—	—	+	tr.	tr.	tr.	tr.	tr.	
Heat-coag. protein..	20	75	8	4	4	1	164	1	128	1	97	1	191	+	tr.	tr.	0	tr.	sl.	
Protein No. 3, whole...	18	75	8	2	6	2	112	4	76	—	—	—	—	+	+	tr.	tr.	tr.	0	
Prim. proteose.....	18	60	8	5	3	2	26	1	112	—	—	—	—	+	tr.	tr.	tr.	tr.	0	
Second Proteose....	11	39	8	6	2	—	—	2	100	—	—	—	—	+	tr.	tr.	tr.	tr.	0	
Heat-coag. protein..	11	39	8	4	4	4	104	—	—	—	—	—	—	+	+	0	0	tr.	0	
Peptone.....	15	32	8	6	2	—	106	1	55	—	—	—	—	0	0	0	0	0	0	
Protein No. 4, whole...	11	39	8	7	1	1	41	—	—	—	—	—	—	+	tr.	tr.	tr.	0	0	Probably prior spontaneous infection
Lipoids, Neutral Fats.	11	36	4	2	1	1	148	1	161	—	—	—	—	+	+	tr.	tr.	0	sl.	
Fatty Acids.....	11	21	4	2	2	2	81	—	—	—	—	—	—	sl.	0	0	sl.	0	0	
Ether-Fats.....	10	29	4	1	3	3	79	—	—	—	—	—	—	+	0	0	+	0	0	
Alcohol-Fats.....	8	4	4	1	3	—	—	—	—	1	53	2	237	+	+	+	0	+	marked	

Combination of Protein No. 2 Protein No. 4 Alcohol-Fats...	4	75	8	3	5	—	—	1	98	1	94	3	106	+	+	+	0	+	—	
Other Preparations:																				
T.B. Emulsion, Koch	20	118	4	1	3	3	86	—	—	—	—	—	—	+	+	tr.	tr	tr.	tr.	
" von Ruck	12	45	4	2	2	—	—	2	149	—	—	—	—	+	+	+	+	+	sl.	
Old Tuberculin, Koch.	20	7cc.	4	2	2	—	—	2	106	—	—	—	—	+	tr.	tr.	tr.	tr.	0	
Bouillon Filtré, Denys.	18	7cc.	4	2	2	2	68	—	—	—	—	—	—	1.	0	0	0	0	0	
I. K. Spengler	14	65	4	3	1	1	54	—	—	—	—	—	—	0	0	0	0	0	0	Probably prior spontaneous infection
Sol. of T. B. (Much):																				
Formic Acid.....	15	50	4	2	2	1	66	1	39	—	—	—	—	+	tr.	0	tr.	0	tr.	
Sod. Glyco	15	50	4	2	2	2	68	—	—	—	—	—	—	tr.	0	0	0	0	0	
Lactic Acid.....	18	60	4	2	2	1	128	1	62	—	—	—	—	tr.	tr.	0	0	0	tr.	
Salicyl. Acid.....	15	50	4	1	3	1	204	2	104	—	—	—	—	+	+	tr.	tr.	tr.	sl.	1 animal had only lymph gld. tbc.
Controls.....	—	—	24	7	17	7	75	10	75	—	—	—	—	—	—	—	—	—	—	2 animals had only abdominal tbc.

COMPARATIVE RESULTS OBTAINED IN IMMUNIZATION—EXPERIMENTS UPON

Rabbit No.	Treatment: Preparation	Treatment: No. of Doses	Treatment: Total Mgm.	Complement-fixation: Antigens	Lysis	Infection: Dose Culture	Infection: Mode	Weight
						Mgr.		Gm.
96	Alcohol-fats	25	120	T.B., 1:4; Alcoh.-Fats, 1:4; Prot. β2, 1:4; Others, tr.	tr.	0.01 "	i. p.	2070
220	" "	12	85	T.B., 1:4; Alcoh.-Fats, 1:4; Prot. β2, 1:4; Others, tr.	tr.	" "	"	2420
168	Ether-fats	11	49.5	Tr. with all antigens	tr.	" "	"	2000
172	" "	16	96.7	" " " "	tr.	" "	s. c.	2580
66	Primary Proteose from Protein β2	12	106	T.B., 1:4; Prim. Prot., 1:4; all others, tr.	tr.	0.01 "	i. p.	2260
67	" " "	15	147	Prim. Prot., 1:4; all others, tr.	tr.	0.02 "	s. c.	1720
159	Secondary Proteose from Protein β2	16	83.6	Sec. Prot., 1:4; T.B., tr.; others, negative	0	" "	i. p.	1700
68	" " "	15	112.3	Sec. Prot., 1:4; others, tr.	0	" "	"	1900
72	Heat-coag. Prot. from Protein β1	10	49	Heat-coag. Prot., 1:4; others negative	0	" "	"	2000
156	" " "	10	46.9	Same as β 72	0	" "	"	2500
176	Nucleo-Protein	12	61.2	All antigens, tr.	0	0.01 "	s. c.	2270
152	" "	11	52	T.B., 1:4; Nucl.-Prot., 1:4; others, tr.	0	0.02 "	i. p.	2000
177	" "	11	55.9	Same as β 152	0	" "	s. c.	2700
79	Prim. and Sec. Proteose from Protein β1	14	144	Prot. β1, 1:4; others, negative	0	" "	i. p.	2270
87	Wat. Ext. T.B.	15	200	Not tested	—	" "	"	3700
116	" " "	12	118.2	T.B. and Prot. β 2, 1:4; lipoids, tr.	0	" "	"	2230
103	" with heat-coag. prot. removed	12	128.7	All antigens, tr.	0	" "	"	2400
153	" w. fats removed	16	97	" " "	slight	" "	"	2100
79	Peptone from Prot. β 2	14	144	Proteins, tr.	0	" "	"	2270
77	" " " "	11	60	Negative	0	" "	"	2700
202	Old tuberculin	14	8.4 c. c.	All antigens, tr.	0	0.02 "	s. c.	2900
149	" "	16	10.2 c. c.	Old tuberculin, tr.; others negative	0	" "	"	2110
120	Albumose-free tuberculin	15	7 c. c.	A-F., tuberculin pos.; T.B. pos.; Alcoh. and ether-fats, tr.; Prot., neg.	tr.	" "	"	2030
122	" " "	13	23 c. c.	A-F., tuberculin and T.B., tr.; others, neg.	tr.	" "	"	1750
49B	Endotin	17	25 c. c.	Endotin and T.B., tr.; others, neg.	0	" "	"	2480
123	"			Same as β 49B	0	" "	"	2020

RABBITS WITH PARTIAL ANTIGENS AND WITH OTHER PREPARATIONS

*) + = positive
0 = negative
— = not made

Autopsy									Remarks
Weight	Infection Site	Reg. L. Glands	Internal Organs	Pseudo	Cause of Death	Lived Days	*) Smears	*) Sections	
Gm.									
3180	fibrous	0	Few hard nodules in intestine, kidneys and lungs	0	killed	100	+	0	Abscess at site of infect. of emuls.
2600	0	mes. gld. enl.	Normal	0	"	97	0	+	Mesent. gld. histol. tubercles.
1520	scar	0	"	0	trauma sepsis	37	—	—	
3100	caseous	caseous	Few tubercles in diaphragm; many fibrocas. foci; 1–5 m.m. in lungs	lungs ?	killed	100	+	+	Calcareous foci in lung.
2130	"	0	Indurat. area, right apex; both lungs many hard nodules	lungs	"	122	+	+	
1620	scar	caseous	Globular pearl tumor attached to diaphragm, caseating tubercles in lung	0	"	99	+	+	
1880	0	0	Several nodules w. centr. caseation in lung; two calcar. nodules in omentum	0	"	98	+	+	
1530	0	0	Recent tubercles and several large, white necrotic areas in liver	liver	no cause	42	+	+	
2300	caseous	caseous	Generalised tuberculosis	0	tbc.	59	+	+	
1250	"	"	" "	0	"	65	+	+	
2400	"	"	" "	0	"	64	+	+	
1600	scar	0	Tbc. of both lungs	0	killed	97	+	+	
2920	caseous	0	Tbc. of both lungs; several nodules in kidneys	0	"	99	+	+	
2520	"	0	Few non-caseating tubercles in mesentery and omentum, small intest. diaphragm, lungs	0	"	97	+	+	
3690	0	0	4 suspected foci; 2 m.m., non-cas. indurations in lungs	lung ?	"	104	0	0	
2300	caseous	enlarg.	Few non-cas. nodules in rt. test.; and liver; several in lungs	testis liver	"	97	0	lung +	
2780	fibrous indur.	0	6 nodules, 6 m.m., in each kidney; numerous hard nodules in lungs; 3–5 m.m.	lungs kidneys	"	98	0	0	
2200	scar	0	2 nodules in left suprarenal; 2 m.m.; fibrous area, 5 m.m. in right lung	0	"	97	lung +	lung +	Fibrous tubercles in lung, (histol.).
2550	caseous	caseous	Generalised tuberculosis	0	"	97	+	+	
2920	"	"	" "	0	"	99	+	+	
2500	"	0	Chronic caseating tbc. of both lungs and kidneys	0	"	99	+	+	
1800	"	caseous	Chronic caseating tbc. of lungs, kidneys and intestine	0	"	89	+	+	
1480	"	"	Generalised tuberculosis	0	tbc.	115	+	+	
1300	"	"	" "	0	"	85	+	+	
1400	"	"	" "	0	"	90	+	+	
1360	"	"	" "	0	"	52	+	+	

COMPARATIVE RESULTS OBTAINED IN IMMUNIZATION—EXPERIMENTS UPON

Rabbit No.	Treatment			Complement-fixation	Lysis	Infection		
	Preparation	No. of Doses	Total Mgrs.	Antigens		Dose Culture	Mode	Weight
						Mgr.		*Gm.*
52	Lactic acid sol. of T.B. (Much)	17	130	All antigens, posit.	tr.	0.02 bov.	i. p.	1930
53	" " "	17	130	Same as # 52	"	" "	"	2690
47B	Tuberculo-mucine (Weleminsky)	12	176	Tubere.-muc., 1:4; T.B. 1:4; others, tr.	"	0.01 "	"	2500
196	" " "	13	50	Tubere.-muc., 1:4; all others, tr.	0	0.02 "	"	1810
200	" " "	17	200	Tubere.-muc., 1:4; T.B., 1:4; all others, tr.	tr.	0.01 "	"	1860
55	T.B. Emulsion, Koch	40	105	T.B. emuls. and T.B., tr.; others, neg.	"	" "	"	1980
225	" " "	17	50	Same as # 55	0	" "	"	1900
232	Living avirul. T.B.	17	78	Negative all antigens	0	0.02 "	"	2400
207	" " "	11	50	Same as # 232	0	" "	"	2500
64	Dead T.B.	11	119.5	T.B., 1:4; alcoh.-fats and prot., tr.; other fats, neg.	0	0.01 "	s. c.	2200
166	" "	15	93.8	T.B., 1:4; T.B. and alcoh.-fats, tr.; ether-fats, neg.; prot., 1:4	tr.	" "	"	1770
191	Bouillon Filtré	15	81	Trace with bouillon filtré and prot.; other antigens, neg.	0	" "	subc.	2400
234	" "	16	126	Same as # 191	0	" "	"	2000
131	Emuls. of Lecithin	16	129	Trace w. lecithin; all other antigens, neg.	0	0.02 "	i. p.	1950
133	" " "	12	85	Same as # 131	0	0.01 "	subc.	2400
140	" " "	11	67	Neg. all antigens	0	" "	"	2530
126	" " Cholesterin	16	90	" " "	0	" "	i. p.	2100
135	" " "	17	127	" " "	0	" "	"	2270
141	" " "	16	97	" " "	0	" "	"	2250
117	Sterile Bouillon	18	125	" " "	0	" "	subc.	2790
154	" "	16	100	Trace w. bouillon Same as # 117	0	" "	i. p.	2140

RABBITS WITH PARTIAL ANTIGENS AND WITH OTHER PREPARATIONS—*Continued*

*) + = positive
0 = negative
— = not made

Weight	Autopsy: Infection Site	Reg. L. Glands	Internal Organs	Pseudo	Cause of Death	Lived Days	*) Smears	*) Sections	Remarks
Gm.									
1700	caseous	caseous	Generalised tuberculosis	0	tbc.	165	+	+	
1900	0	enlarg. fibrous		0	no cause	177	0	0	
2680	caseous	0	Pearl tumors in periton., oment., liver; caseat. tubercles in mesentery and lung	0	killed	96	+	+	
2070	"	0	1 nodule, 1 m.m. in each kidney; many caseating foci in lungs	lungs	"	97	0	lt. lung + rt. lung 0	
2880	"	enlarg.	Few fibrous nodules in kidneys; numerous in lungs	lungs	"	96	infect. site	doubtf.	
1940	"	0	Numerous tubercles in omentum, liver, kidneys; many fibrocas. foci in both lungs; necrotic foci in liver	liver	"	96	+	+	
1100	"	caseous	Generalised tuberculosis	0	tbc.	50	+	+	
2280	0	"	Several 2 m.m. nodules in mesent., oment., kidneys, liver, diaphragm; lungs full of recent tubercles	0	killed	92	+	+	G. P. No. 2325 infect. with emuls. from lung; 500 gms; died 161 days; 400 gms., gen. tbc.
1560	caseous	"	Generalised tuberculosis	0	tbc.	72	+	+	
2000	"	"	" "	0	killed	96	+	+	
1530	"	0	Large fibrocaseous foci in both lungs	0	no cause	35	+	+	
1980	"	caseous	Miliary tuberculosis of liver, spleen, mesentery, omentum; many fibro cas. foci in lungs, diaphragm	0	tbc.	76	+	+	
			Generalised tuberculosis	0	"	72	+	+	
CONTROLS									
1150	caseous	caseous	Generalised tuberculosis	Pseudo liver and lung	tbc.	47	+	+	
1640	"	"	" "	0	killed	97	+	+	
1940	"	"	" "	0	"	96	+	+	
1850	"	"	Numerous fibrocas. foci in lungs	0	tbc.	131	+	+	
1530	"	"	Generalised tuberculosis	0	"	127	+	+	
1930	"	"	" "	0	"	70	+	+	
1680	"	"	" "	0	"	123	+	+	
1640	"	"	" "	0	"	70	+	+	

found marked positive reactions with several proteins, except in animals which had been treated with our ether-extractive and with Much's Fatty Acids. Much prepares his lipoid antigens by first saponifying the tubercle bacilli and then extracting the soap with alcohol and with ether. The same solvents are used by us for obtaining our lipoid extractives. With both these methods some proteins are apt to be present in the extractives, especially in those obtained with alcohol. It appears, therefore, that the lipoid antigens, both those of Much and our own, are not assuredly free from proteins, and the positive results in complement-fixation, with the sera of animals treated with them, might be due to the presence of these proteins in the antigens.

In controls treated with lecithin and cholesterin we found that, with the sera of animals treated with the former, we could obtain positive results in complement-fixation with lipoids from tubercle bacilli as antigens, while this was not the case with sera from animals which had been treated with cholesterin. This observation caused us to examine human sera, obtained before and after treatment with vaccine, using lecithin and cholesterin as antigens, and we found that complement-fixation was positive with lecithin with sufficient frequency to raise the question whether or not the reaction observed with lipoids from tubercle bacilli was actually a specific one. Unfortunately the lack of normal animals, and the frequent deaths in those which had acquired pseudo- or spontaneous genuine infections, once more made it impossible for us to complete our studies which we had contemplated and, on that account, the whole subject was left in doubt. The results of the related animal experiments are given in the accompanying tabulations.

In the records of these experiments upon guinea-pigs, it will be noted that we lost nearly one-half of the animals in the course of treatment, or before sufficient time had elapsed after infection, to justify their consideration. These premature deaths appeared to stand in relation almost entirely to pseudo-tuberculosis or to spontaneous genuine infections. The deaths which occurred from these causes, more or less reduced the numbers of animals treated with certain fractions of the constituents of tubercle bacilli; in the experiments with Protein No. 4 (Neucleoprotein) we lost all but one animal.

Although we had hoped that previously diseased animals were practically eliminated from the lot under experiment and that all had died during treatment, there were still twenty-four guinea-pigs which showed pseudo-tuberculosis on autopsy, and several which had old genuine lesions that antedated our infection.

The irregular numbers of the groups that were available for final consideration, the difficulties in differentiating between genuine and pseudo-tubercle, and between spontaneously acquired and our artificial infections, when they were associated in the same organ or in the same lesion, were so great that it was impossible to determine our findings with absolute certainty in every instance, and this detracts from the value of the results.

Rabbits appear to suffer less frequently from pseudo-tuberculosis and, in case they do, they are less liable to die under specific treatment or soon after infection with tubercle bacilli; but we lost a considerable number of these animals from pneumonia, coccidiosis and other causes. To a certain degree the experiments upon guinea-pigs and upon rabbits seem, however, to support each other in their results.

As we consider the experiments made on rabbits on the whole to be more satisfactory, we have tabulated them individually, but grouped the experiments on guinea-pigs in order to save space.

The tabulation of experiments on guinea-pigs shows that all of the seven animals, which had been treated with the whole vaccine, resisted the infection completely, and that a like success was obtained in six of the seven guinea-pigs treated with Watery Extract, in three out of four animals treated with a heat-coagulable albumin of the same protein, in three animals treated with a combination of Protein No. 2, No. 4 and an alcohol-extractive of tubercle bacilli, and finally in the three animals treated with the same alcohol-extractive alone.

Of interest appears to us the difference in results shown between the heat-coagulable albumin obtained from Protein No. 2 and that from Protein No. 3, and further the fact that the rabbits which were treated with Protein No. 2 and with the alcoholic extract appear to confirm the results obtained in guinea-pigs with the same preparations.

In the experiments with other specific preparations we ob-

tained the best results from the treatment of rabbits with Much's and Weleminsky's preparations; the outcome was perfect in one of the rabbits treated with Much's lactic acid solution. Rabbits Nos. 196 and 200, which were treated with the Tuberculomucine of Weleminsky, had pseudo-tuberculosis of the lungs, and this obscured the results of macroscopic and of histological examination. In the latter, the lesion of the right lung showed tubercles without giant-cells, but no tubercle bacilli were found in the antiformin residue of tissue from the same lesion.

The observations in complement-fixation test are also of interest in that it was positive with the antigens used for the treatment of the animals, with the exception of the pepton from Proteins Nos. 1 and 3. As a rule, the treatment had been continued until complement-fixation had become positive. Of special interest are the results obtained in complement-fixation tests of sera from animals that had been treated with the alcohol-extracts from tubercle bacilli, which in the guinea-pigs were also positive with specific proteins, although theoretically none of the latter should be present in the antigen used for treatment.

In whatever manner these observations will ultimately find an explanation, at present they are not sufficiently clear to settle the relative importance of the several partial antigens in experimental immunization definitely. Before we make further experiments, we shall endeavor to obtain our antigens and their fractions in a purer form, and to secure animals in which we do not have to reckon with pseudo- and spontaneous genuine tuberculosis.

For the demonstration of the additional specific component in the culture-medium, and of its exo- or endo-toxic origin, as well as that of our Protein No. 1, special culture-media are required as a first step before others can be taken, and before biological reactions in normal animals can be of decided value.

In relation to an additional specific component of tuberculin, it may be of interest to revert to the question whether, during its life, the tubercle bacillus produces a ferment-like toxin that is not of protein-nature and may be found in the culture-media, and to the further question of the presence of a specific ferment in the body-substance of the bacillus. We have in mind certain

experiments which we made in 1895, when we standardized old tuberculin for its toxic action upon tuberculous guinea-pigs, the requirement being that 0.5 cc. of tuberculin must kill, in 24 to 48 hours, such an animal which had been infected a certain time before with virulent tubercle bacilli. When we added an equal amount of fresh serum from a previously immunized animal, the expected death did not occur and some of the animals were not even made sick. Evidently we had neutralized some toxic substance, which is responsible for the fatal reaction, with some antibody contained in the immune serum, and this antibody seemed dissimilar from a true antitoxin only in so far as it required so large an amount of the serum to neutralize the toxin. The protection seemed, however, to depend upon a similar reaction as that between a true toxin and its antitoxin, and even now we cannot think that the large quantity of 500 mgr. of tuberculin could have been reduced completely and destroyed by the bacteriolytic amboceptors and complement contained in one-half cubic centimeter of the serum. Since then like experiences have been recorded by other observers, and among them by Van Calcar,[1] who immunized his animals with what he believes to be a non-proteid substance extracted from serum-agar upon which virulent tubercle bacilli have been grown and which had been removed carefully. This substance he considers to be a true toxin. In the light of Van Calcar's observation, this question deserves further inquiry. We are, however, inclined to the opinion that the component of tuberculin, which caused the diagnostic reactions, is a proteid substance.

In our own studies of the evolution of naturally acquired immunity in the course of tuberculous disease, we have accumulated numerous data which appear to indicate that the immunity is acquired very slowly and by successive steps. The reaction of the organism to tuberculin constitutes the first clinical evidence of a tuberculous process, and we believe that it is caused, at least in part, by the highly soluble Protein No. 1 which we have described. A specific reaction can be produced with tuberculin and, in very early cases, also with this protein, often at a time when the injection of other products of the tubercle bacillus still fails to elicit a response. A reaction to any particular product of the tubercle bacillus indicates that the organism does

[1] Van Calcar; *loc. cit.*, S. 126 (p. 168).

not yet possess a sufficient amount of lytic amboceptors, specific for it, to reduce the injected dose completely or at least sufficiently to prevent the production and accumulation of split products which cause the irritation at the site of the injection, especially when the toxin can be absorbed but slowly, as is the case in the percutaneous test. It seems, however, that the amboceptors in the blood are sufficiently numerous for a complete reduction of the very minute amount which may be absorbed, because a general reaction does not occur. After the administration of a small subcutaneous dose, however, the general and focal reactions show that the specific amboceptors in the blood are below the maximum. These reactions may be increased or prolonged by repeating a subcutaneous dose of tuberculin before the first reaction has subsided, and the same appears to take place in acute miliary tuberculosis, when a large amount of a toxin is constantly absorbed and is then continuously present in excess in the circulation. Inasmuch as the tuberculin test is negative in acute miliary tuberculosis, the conclusion is justified that the toxin, which is present in excess, is similar in nature to the one in tuberculin.

Clinically we find that, although reactions no longer occur to larger doses of tuberculin, even very minute doses of one or the other of the bacillary body-substances can still produce local, general and focal reactions, and this is often observed most readily with a lipoid. These reactions disappear as the doses are repeated. The sequences and observed results of gradual successive and steplike immunization with different fractions of the products of tubercle bacilli have been obscured frequently in our studies by the fact that most of our fractions were more or less of mixtures with one or more other fractions. We have, however, been able to complete an existing partial immunity in the human subject and in animals, as Much has done, and to cause the development of the missing specific partial amboceptors, by administering the related fractions.

We are fully convinced that the further study of these questions is of the greatest importance, and that only by their full elucidation may we expect ultimately to explain certain experimental and clinical observations, the causes of which are still obscure. Personally we feel certain of the existence of a constituent in the tubercle bacillus, which is not necessarily a pro-

tein or a lipoid, although most likely intimately associated with one of them, probably with one of the lipoids. We believe that it is this constituent which is responsible for the formation of a specific thermolabile antibody of ferment-nature, upon which the bacteriolytic and germicidal actions of a given serum depend. We have also reason to believe that it is this substance which stands in relation to the autolytic changes which were observed in our vaccine when it was kept at room-temperature, and that, until we can determine its presence quantitatively by proper tests, we must reckon with the possibility that the amounts in our vaccine may vary in different lots, although the general mode of manufacture remains the same.

Our observations, mentioned on p. 94, that a given human or animal immune serum which, in its active stage, had caused marked and unmistakable morphological changes in tubercle bacilli *in vitro,* could not be reactivated with fresh normal human or animal serum, and that the tubercle bacilli were in consequence not deprived of their virulence when they were incubated with serum-specimens before they were used for infection, points unmistakably to a specific thermolabile substance in immune serum which is not present in appreciable quantities in normal sera.

BIOLOGICAL AND EXPERIMENTAL STUDIES

The clinical results which were observed in the course of the prophylactic use of our vaccine, and which have been described in a preceding chapter, could not have been anticipated when we first undertook our studies several years ago, and we should not have been disappointed if additional treatment had been required in cases in which the infection had advanced to such a degree that structural changes were demonstrable on physical examination, in order to stimulate and cause their retrogression or disappearance.

In the expectation that the prophylactic administration of vaccine would be followed on the part of the organism by the development of specific bacteriolytic antibodies against the tubercle bacillus and that, in this event, infection would be resisted more or less, also that it would be checked in case it was already

acquired, we took pains to examine the sera of all persons whom we vaccinated, for such antibodies. When no exceptions were found in this respect, and the titer of the sera seemed to be maximal in amounts, we believed the view justified that the vaccinated children had acquired an increase of their defenses against the bacillus of tuberculosis, because these antibodies are specific; and it was our purpose to recommend vaccination on that account alone, even had we not succeeded in the demonstration of a bactericidal power of the sera after subjecting virulent tubercle bacilli to their action outside of the living body, in the test-tube. We had planned to present our results, several months before the date on which we reported them before the Chicago Medical Society on May 1, 1912, and we delayed our communication purposely because we desired further time in order to assure ourselves of the correctness of our bactericidal results.

The reader will no doubt appreciate that such a test is an extremely severe one, and that the specific resistance of the living organism could be sufficient in degree even though it were not demonstrable by the destruction or reduction of virulence in the course of a few hours after incubating a virulent dose of tubercle bacilli with a few drops of the serum. It must be assumed that the living human organism may and does accomplish this destruction more surely, since it has many thousand times the amount of specific antibodies at its disposal at any one time, which moreover are subject to constant renewal during the entire time required for the growth and multiplication of tubercle bacilli which might gain or have gained entrance to the tissues.

In reporting now upon the results of our further investigations, we may be permitted to save the space required for similar tabulations of our findings of specific antibodies, as they were incorporated in our report of 1912, because we obtained practically identical results until certain technical difficulties were encountered which were again overcome in the end. In view of the fact that these difficulties are likely to arise in similar studies of other observers, and that their understanding is necessary for the critical examination of our own work, we deem it important to relate them in detail.

It should be borne in mind that the application, by us, of

the complement-fixation test, and the comparative determination of morphological changes caused by sera in living virulent tubercle bacilli before and after the administration of the vaccine, had the purpose of showing that a response with specific amboceptors has occurred to the administration of our vaccine and of supplying us with a guide which could indicate that germicidal substances were present in sufficient amounts to justify the assumption that the virulence of tubercle bacilli would be destroyed in the living organism.

The question of positive results in complement-fixation, in response to the parenteral administration of a foreign proteid, tissue-cell or bacterium, was never a matter of trial, neither was the bacteriolytic and germicidal action of the related amboceptors a subject which it was incumbent upon us to establish as one of the principles in active immunization. We did not even have to supply any proof that lower animals can be immunized against the pathogenic action of the tubercle bacillus. All these questions had been settled authoritatively by others, and all that we did was to apply the principles determined by them to our own studies in tuberculosis.

The possibility of immunizing large animals against the tubercle bacillus was demonstrated years ago by v. Behring and others, and received confirmation recently by Gilliland's experiments with dead tubercle bacilli. We ourselves, and numerous other observers, have reported successful results in like experiments upon guinea-pigs.

Our particular efforts were directed to the production of a soluble vaccine for use in human subjects, in which the bodies of the bacilli were excluded and their endotoxins in solution substituted in a form in which they are not injured chemically or by heat. If we had failed to obtain positive results, the explanation would have had to be found in our technical inability, or in a difference between the vaccine which we administered and the antigens against which we tested the sera, provided the subjects supplying the latter had been treated with them sufficiently; unless tubercle bacilli or their body-substances should prove an exception to most, if not all, observations of immunity-reactions. That this was not the case was shown by many others who obtained positive reactions, and we recall the fact that the tubercle bacillus was the first bacterium, by the

study of which the complement-fixation test became applicable for the practical demonstration of specific amboceptors, by Bordet & Gengou.

The only observation on our part, which is not recognized generally, is the occurrence of marked morphological changes in tubercle bacilli when they are exposed to the action of fresh immune serum in the test-tube. Similar results have, however, been described by Dembinski,[2] Van Calcar,[3] Rosenberg[4] and by others. Dr. Chas. C. Browning, of Los Angeles, California, who studied the subject for nearly two years in a considerable number of patients who were being treated with our vaccine. informs us in a personal communication that he has obtained results which correspond with ours in all respects. These morphological changes, and the tests in complement-fixation, have served us as a guide for the degree of bactericidal action of immune sera, and they have proved sufficiently reliable in our studies to justify their adoption, as will become evident in the records of the related experiments.[5]

It is self-evident that some sort of a guide is desirable before proceeding with the infections of animals; if one had been available years ago, to us or to other students who attempted to solve the question of specific bacteriolytic immunity in tuberculosis, we believe that much groping in the dark could have been saved, and more uniform results would have been obtained; at the same time the useless sacrifice of animals, and considerable incidental expense, could have been avoided in purchase, care and treatment.

The variations which were noted in the amboceptor-titer of

[2] DEMBINSKI; Zeitschr. f. Tuberkulose. 1907, X, 423.

[3] VAN CALCAR; *loc. cit.*, S. 194 (p. 168).

[4] ROSENBERG; Russky Wratsch; 1911, No. 30. Cf. Fol. Serol.; 1911, VII, 163.

[5] The comparative studies require much time and patience, and we call particular attention to our observation that, under the action of a fresh immune serum, the tinctorial properties of tubercle bacilli are likely to be modified, in that any existing rods and granular detritus may retain the carbol-fuchsin stain only indifferently or not at all, especially when higher per cents of mineral acids have been used for decoloration, and that then they may take the counter-stain. It is therefore advisable to examine and to compare series of slides made from the same specimen, in which different percentages of acid have been used for decoloration.

sera taken before and after vaccination, and during and after treatment, made it necessary to examine the sera again if the desired titer was not present on the first trial. Although this was troublesome, especially in small animals like guinea-pigs, we felt that we were amply compensated in attaining uniform results in consequence. When in these small animals several successive tests proved unsatisfactory, or when we feared that the further abstraction of blood might be injurious to them, we either allowed them to rest for ten days or two weeks and then tried again, or we gave them a few additional doses of vaccine before repeating the trial; in following this course we had no failures. Although we observed successes in some cases in which our standard had not been complied with fully, we did not lower it on that account, but reserved the study of the real MINIMUM essential for success, for a later and more opportune time. It was also necessary to ascertain how many and how large doses of vaccine were required in order that the immune sera should come up to the quantitative standard for antibodies in every case.

These questions having all been answered satisfactorily, we accepted our standard as sufficiently high to enable us to avoid failures. It could be demonstrated to have been attained in the human subject after one full dose of vaccine; very often this was the case upon the first test; it was so as a rule on the second or third test; while we were not unmindful of the possible chance that the result might be influenced by technical conditions and variations so slight that we remained unaware of them, we did not propose to take any chances and preferred to repeat the tests.

As we were interested in the practicability of our method for prophylactic purposes, it was necessary to show sufficiently uniform results, without which some doubt would have been left concerning the value of the vaccination in the individual case; further, the effect of the vaccination had to be of sufficient duration, since the practicability of the method depended also upon the frequency with which the vaccination would need to be repeated. Inasmuch as we could not demonstrate by actual experiment that the human organism would resist infection after vaccination, it was necessary to apply our method upon susceptible animals and to find whether the same guide would

enable us to show that their resistance was complete and that, like in the human subject, their sera acquired bactericidal power. We freely admit that in following our guide we met with occasional technical difficulties, but when a reaction with a given serum specimen or with a number of specimens was unsatisfactory on a given day, or when our results were left in doubt, there was nothing to hinder us from repeating the work as many times as we saw fit and as long as our patients were willing to supply the blood.

In continuing our studies we desired reliable answers, among others, to the following questions:

(1) Can specific amboceptors and lysins be demonstrated in all animal and human sera after vaccination? Do they occur in sera obtained before vaccination and, if so, what is the relative frequency of this occurrence?

(2) Is there a quantitative relation to specific resistance and, if so, what is the minimum requirement in order that the animal supplying the serum shall resist an infection, and in order that human sera shall cause the destruction of virulence of tubercle bacilli?

(3) For how long a period after vaccination can these results be obtained, and does the size or a repetition of a dose have an influence upon the length of this period?

A study of the experimental data, which will be given further on, will enable those interested to judge in how far we have been able to obtain reliable answers, and in how far these answers support the practical observations made by us and by others, of the value of our methods. The publication of the results obtained independently in the laboratory of Sir Almroth E. Wright in London has been unavoidably delayed, but it is expected at no distant future. While it is not for us to discuss these results at this time, we have sufficiently complete information concerning them to enable us to state that, judging from the copies of the autopsy protocols, we are entirely satisfied with them. In regard to our own biological studies, our results have been confirmed especially by those who have taken the trouble to familiarize themselves with our methods, and in this respect we refer particularly to the publication by Terrill,[6] because, according to his own statement, he approached the subject

[6] J. J. TERRILL; *loc. cit.* (p. 138).

doubtingly, but felt constrained to confirm us after a painstaking investigation.

With the practical results of prophylactic vaccination, which we now have before us, and which have been and are being confirmed daily at the hands of others, the actual and visible benefits referable to vaccination against the tubercle bacillus are available sufficiently to decide upon the practical value of our method, and, in consequence, both the serum-tests and the animal experiments have diminished in importance. In the last instance it makes no difference whether these benefits actually arise through the production of an artificial immunity with or without the development of specific amboceptors or lysins, since the relation of the vaccine to the practical clinical results has been established.

These experiments were and are still of importance, however, in respect to the proposed vaccination of normal, i.e., not infected children, for the purpose of determining the correctness or the error of those authors who held that normal human subjects cannot be immunized against the bacillus of tuberculosis.

This assertion that normal, that is to say, not infected, human subjects cannot be immunized against the tubercle bacillus seems illogical and irrational, in the light of acquired immunity to infectious diseases, and in view of the process of autoimmunization which is postulated with so much reason as occurring spontaneously in every person who, after a first infection with tubercle bacilli, develops a degree of immunity and thereafter fails to acquire clinical tuberculous disease, by opposing a sufficient immunizing response to every renewed infection, for the reason that the first infection had sensitized the organism, though it had not led to disease. Even if living tubercle bacilli are used for the immunization of normal noninfected calves, as in v. Behring's method, an immunizing response does not occur until the injected tubercle bacilli have been broken down and disintegrated. It is self-evident that, if this breaking-down and disintegration is accomplished outside of the body and if the resulting bacillary constituents are injected in solution, the immunizing response must become operative at once. Observations confirming this view have recently been published by A. Jousset, in the Bulletin of the Academy of Medicine of Paris.

DIFFICULTIES IN THE COMPLEMENT-FIXATION TEST

Quantitative complement-fixation tests have been a routine procedure in our laboratory since the winter of 1907-08. The technic used in them differed from the original one of Wassermann chiefly in that we used only 0.05 cc. of a serum-dilution of 1:4 in physiological salt solution, and in other slight modifications which our preliminary studies had shown to be best suited for our study of immunity in tuberculosis. We had little or no difficulty, certainly never more than must be expected in such complex biological reactions. During the summer of 1913, however, difficulties arose more frequently and in time they became annoying. At first we were inclined to attribute them to the use of serum from diseased animals, for complement, since we found at that time that pseudo- and genuine tuberculosis was prevalent among our supposedly normal animals.

This view was seemingly no longer tenable when some of the animals supplying complement had been bled to death and when on autopsy no evidence of disease was found in them. A study of our hemolytic system, and various changes made in it, and in other technical details likewise failed to give us the former uniform results; yet we succeeded often enough in obtaining unobjectionable reactions that we could continue to make use of the tests. In July of 1914, however, the failures became so frequent (and had, moreover, extended to tests for agglutinins and precipitins) that all routine serum tests had to be abandoned, while our efforts to find the cause or causes of our failures were of course continued.

We satisfied ourselves that the difficulties were not attributable to the hemolytic system, nor to the technic employed by us. The failure in the agglutinin and precipitin reactions caused us to look for the origin of the trouble in the antigens which had always been prepared from the same strain of tubercle bacilli, grown in our laboratory since 1901, and which had never before failed us. In November, 1914, we were in a position to study the action of our antigens comparatively in regard to the influence of age, using a supply which had been made in June, side by side with a new lot which had just been finished; both lots were entirely unsatisfactory, and there was

no difference between them; this appeared to eliminate the factor of deterioration of the antigen by age.

When one of the writers went to London, in June, 1914, in order to be present at the final testing of animals treated with our vaccine, and of human immune sera taken before and after prophylactic vaccination with the same preparation, he took with him a supply of antigens prepared immediately before his departure. After his arrival in London, a small lot of treated guinea-pigs was tested and the sera of nine persons, to be vaccinated, were examined with the old antigens which had been sent to London the preceding February, both without much difficulty. The supply of the old antigens which had been used in these tests was then exhausted, and the new antigens, made in June, were used in further tests; but with them it was impossible to obtain clear-cut reactions, in spite of repeated trials.

The culture from which our antigens were prepared had been examined frequently during preceding years without marked changes being detected. On examining it again, in November, 1914, its growth was still found to be typical of that of a human culture upon agar and upon bouillon; but to our surprise it had changed greatly in its morphological appearance, there being now only few typical rods. Instead of these, we found extremely short forms, resembling fragments or even cocci and granules, which showed a greatly diminished resistance to acids. An increase of over 5 per cent sulphuric acid in the decolorizing fluid was sufficient to cause the RETENTION OF THE COUNTERSTAIN in most of the organisms and, with 25 per cent acid, in all coccus- and granule-forms; only a few of the rod-forms were still acid-fast.

Here, then, was a degeneration for which we were at first at a loss to account. A searching examination brought, we believe, a most interesting explanation in the discovery of entirely unexpected changes in the chemical composition of the bouillon upon which the culture was grown; this bouillon showed the presence of copper, evidently derived from defective tinning of the large copper vessel in which the bouillon had been boiled when it was needed in large quantities. The tubercle bacilli themselves contained copper in greater amount than did the bouillon upon which they were grown. When our pend-

ing studies of the relation of copper to growth-energy and virulence of tubercle bacilli are completed, the results will be published in a separate communication. After planting the antiformin residue from the degenerated culture upon agar, we now find once more nearly if not entirely typical forms which completely resist decoloration with 25 per cent sulphuric acid; but in their sixth generation upon a copper-free medium, the bacilli still fail to give satisfactory results as antigens in complement-fixation and in agglutination tests.

We have now been working for several months past with another culture of tubercle bacilli, and we find no difficulty whatever in obtaining satisfactory reactions with these tubercle bacilli when they are employed as a whole antigen in complement-fixation; we expect that we shall find the fresh partial antigens equally satisfactory when they become available from this culture.

During our investigations we experimented with variously modified technical procedures, as used or recommended by others or as adapted to our use by ourselves. While we succeeded with none of them as long as we employed faulty antigens, all of them now give more or less satisfactory and uniform results with the tubercle bacilli from the new culture. One outcome of these studies was the abandonment of the "drop" method for accurately measured quantities and dilutions, and a reduction of the total quantities of the several components used in the test, by which a great saving has been effected in complement and in hemolytic amboceptor, as well as in the serum that is to be tested.

The reader who has followed the recital of these various experiences will realize that a rather confusing state of affairs was finally found to prevail, and appreciate that its theoretical explanation was naturally modified from time to time as any particular viewpoint forced itself upon our attention as best calculated to throw light on the subject. With the complete records, ordered and tabulated as they now lie before us and therefore comparable, we can understand the probable causes of these experiences far better than we could during their gradual accumulation, and yet even at this time we shall by no means attempt to pass final judgment, but shall defer doing so until we shall have reproduced the same condition deliber-

ately and intentionally, knowing beforehand the exact circumstances with which we are dealing, and taking cognizance of what we now believe to have been the facts.

* * * * *

As stated above, the difficulties in biological tests of serum were associated with an increasing prevalence of pseudo-tuberculosis in our stock of animals, which it was not possible to eliminate, even though we rebuilt and refitted our animal-houses so that they could be disinfected easily; we found the animals infected in larger or smaller numbers with one or the other of the pseudo types, as they were received from dealers or breeders, and in certain shipments we could not discover a single animal which was free from pseudo-tuberculosis when the entire lots were killed and autopsies made within a few days after their arrival. These preexisting infections, which will be discussed in detail in the next chapter, and their dissemination after the animals were received by us, afford now an ample explanation for many anomalous observations. At the time, they aggravated the existing confusion which could not be cleared up until all the related causes were understood.

Studies and investigations of this sort become increasingly difficult and tedious when more than one unknown disturbing element exists; but they enlarge our experiences and our viewpoint and become instructive especially in the course of biological investigations. They also caution us against forming hasty conclusions.

CHAPTER XI

THE VALUE OF THE GUINEA-PIG IN EXPERIMENTS IN TUBERCULOSIS

We have stated that we noted an unusual behavior in some of the animals soon after we resumed our experimental studies on guinea-pigs, in the fall of 1912, while others behaved normally to the same dose of vaccine. After the first or a subsequent dose, some of the animals appeared to be less active than before, and within one or a few days they had lost from 30 to 100 grams in weight. Some of them died within one week; others continued to decline in weight, dying later in a state of extreme emaciation, and it was not possible on autopsy to find a sufficient cause for most of the deaths, except the excessive loss of weight which was, itself, not explained clearly, although certain of the animals had more or less extensive genuine tuberculous lesions in a condition of reactive congestion. The deaths were nevertheless remarkable, in that some of the animals had succumbed, in the course of twenty-four hours, to so small a dose as one milligram of vaccine, whereas five hundred times that amount of old tuberculin is required to bring about the same result in a tuberculous guinea-pig.

A number of observations of genuine spontaneous tuberculosis came to our knowledge in this manner. Some of the animals died after one or more doses of the vaccine, others after they had been infected with tubercle bacilli incubated with immune serum, and in these cases death often took place at a time which was far too short for our infection to have produced the lesions which were found. We then resorted to cutaneous tuberculin tests, in order to weed out the tuberculous animals from our stock, with the results which we have mentioned in another connection (p. 267).

Our attention had previously been directed to lesions which resembled genuine tubercle macroscopically, but in which focal

reactions were missed; no tubercle bacilli could be found in the antiformin residue, and histological examinations were negative. These experiences led to the realization that, in addition to spontaneous genuine tuberculosis, we were confronted with an endemic of pseudo-tuberculosis, and a new problem presented itself with respect to the relation between the pseudo infection and the action of the vaccine in causing marasmus and death in animals which were apparently free from genuine tuberculous infection or disease. Apart from these two complications, many of the animals under experimentation succumbed, in which no manifest cause for death could be discovered, unless it could be attributed to small, often minute lesions that were found in the lungs in the form of areas of induration, apparently chronic in nature. These lesions were so insignificant in extent that it seemed unreasonable to bring them into relation to the marasmus and death which followed the administration of small doses of vaccine or occurred after infections with tubercle bacilli incubated with immune serum. Another unusual observation was that the site of the injection sometimes became hemorrhagic after infection with tubercle bacilli and that abscess occurred much earlier at the infection site than is usual in normal animals. It was remarkable that either pseudo-tuberculosis or the minute lesions in the lungs were always present in the animals that succumbed so promptly, or after a short time in a state of marasmus, and it was noted further that infections made in the course of the control experiments for bactericidal tests, in which the tubercle bacilli had been incubated with normal serum instead of immune serum, as a rule did not cause early death or marasmus, even though the same lesions were found in the animals after they were killed in the course of the experiments.

In our attempts to account for the unforeseen and often surprising phenomena which we observed, we have come to certain conclusions which we formulate in the shape of a theory. We do not attempt to decide at present whether this theory is sufficient to explain our observations, but give it for what it is worth.

Our theory is as follows:

"Pseudo infections in guinea-pigs cause the development of a specific immunity against the related microörganisms, in con-

sequence of which the disease remains localized, and the health of the animal is not disturbed.

"This immunity is interfered with or suspended by the reaction which follows, when the animal is subjected to the influence of toxins that are formed in response to an infection with tubercle bacilli (and probably with other pathogenic bacteria also), or if it receives a parenteral injection of their toxins This occurs by reason of the local and general reaction to the injection of tubercle bacilli or of their toxins, by which the enzymes (complement) are used up, and not enough or none are left available for the defense against the pseudo infection; the pseudo bacteria, therefore, grow and multiply rapidly and enter the blood, through which they are conveyed to the area of local reaction which is in progress. Being all rapid growers, these organisms are responsible also for the hemorrhagic changes and for the early breaking-down of the local tissue at the infection site, while marasmus and death stand in relation to the general pseudo bacillemia."

With the aid of this theory we can explain the fact that the animals frequently appear to remain entirely free from manifest disease, that they may gain in weight and show only insignificant pseudo lesions after accidental death, or more extensive lesions with a marked tendency to fibrosis. We can likewise understand that the toxemia would be greater after the injection of tubercle bacilli incubated with an active immune serum, and after the liberation of anaphylatoxin in consequence of this incubation; also that the same would be the case in animals that had acquired a certain degree of specific immunity before being infected with virulent tubercle bacilli. This affords an explanation for the observation that marasmus and death usually occurred only under these conditions.

Whatever explanation may ultimately turn out to be the true one, we have found these pseudo lesions so frequently in animals purchased from numerous dealers and breeders in this country, and it was met with so constantly in London, that we have been obliged to give up all experimental work with guinea-pigs until we may be able to find a reliable supply of normal animals or, if need be, until we can arrange for the breeding of our own stock.

It is of course possible to control all autopsy findings by

examining smears and sections from the lesions that are found, and we have been careful to do so when it became evident that the macroscopic appearance of the lesions alone could not be depended upon.

In the various types of pseudo-tuberculosis which we observed ourselves we have found that the macroscopic resemblance to genuine tubercle appears to stand in relation to the age of the lesion, and that, in the type in which we observed the slight pulmonary lesions, there is more marked organization in the lesions of apparently longer standing, which are then characterized by peribronchial and perivascular thickening and infiltration, whereas a greater histological resemblance to genuine tubercle is noted when they are recent.

Rabbits are also subject to these pseudo affections, and in these animals we have, in addition, to contend with coccidiosis. We have, however, not found pseudo-tuberculosis to interfere so seriously in rabbits by early deaths, and, except for the necessity of making sections of all or at least of the principal lesions, we can use these animals for infections with tubercle bacilli of bovine origin. Rabbits are, however, not suitable for experiments with the human type, because this is only slightly or not at all virulent for them.

PSEUDO-TUBERCULOSIS

The term pseudo-tuberculosis was first employed by Eberth,[1] who described some lesions in guinea-pigs which were similar in appearance to those of genuine tuberculosis. The principal histological characteristic which differentiated these lesions from true tubercle was the absence of giant-cells; the affection was found to be due to a micrococcus. Later, several types of pseudo-tuberculosis were described by Preisz.[2] We omit from our present consideration the *Tuberculose zoogléique* of the French authors, because it is not relevant to our subject.

One of the three types of pseudo-tuberculosis spoken of by Preisz was that due to the *Bacillus of Pseudo-Tuberculosis of*

[1] C. J. Eberth; Virch. Arch. 1885, C, 15, and 1886, CIII, 488

[2] For literature see K. Grabert, in Kolle & Wassermann; Handbuch der pathogenen Mikroorganismen. Jena (Fischer), III, 1905, 751.

Rodents, first described by A. Pfeiffer.[3] This is a saprophyte which is frequent in nature and acquires pathogenic characteristics accidentally. It is most frequently the virus responsible for the plague of rodents, which is quite common in guinea-pigs. Pfeiffer found that the inoculation of pseudo-tuberculous tissue taken from a horse, caused guinea-pigs to die eight to nine days later. At the point of infection an induration had formed, passing into an ulcer with cheesy, friable pus. The inguinal and mesenteric glands were swollen; the adjacent connective tissue was set with nodules, often in pearl-string arrangement. Like nodules were found in the omental fold between stomach and spleen, also in the spleen itself and in the liver. According to Pfeiffer, the lesions of pseudo-tuberculosis of rodents are characterized by their rapid evolution and by the rapid breaking-down of the pathological tissue; also by the prompt disappearance of the bacilli from the necrotic or caseous tissue. His cultures of the bacilli, on sheep-serum, grew in the form of small drops, slightly opalescent. While the lesions have a close resemblance to those of glanders, Pfeiffer found that the bacillus is quite different from Löffler's Bacillus of Glanders; it stains well with fuchsin and methylene-blue (alkaline), and is Gram negative. Spores were not observed.

Pfeiffer's type of pseudo-tuberculosis of rodents has been studied frequently, more recently by Messerschmidt & Keller,[4] in whose experience it had a more virulent and deleterious character than that of genuine tuberculosis in guinea-pigs when it is acquired by "natural" infection. In the pseudo lesions they found that giant-cells were always, epithelioid cells almost always absent, and that therefore the histological differentiation of pseudo- and of genuine tuberculous lesions presented no difficulty. The constant absence of giant-cells is, however, denied by Apostolopoulos,[5] and also by Kirch,[6] both of whom found them in some of the sections which they examined. Aposto-

[3] A. Pfeiffer; Ueber die bacilläre Pseudotuberkulose der Nagetiere; Leipzig, 1889. Cf. Centrlbl. f. Bakt.; 1890, VII, 219. Also cf. K. Grabert; *loc. cit.*

[4] Th. Messerschmidt & Keller; Zeitschr. f. Hyg., etc. 1914, LXXVII, 289.

[5] G. B. Apostolopoulos; Inaug. Diss. Tübingen, 1893.

[6] E. Kirch; Arch. f. Hyg. 1913, LXXVIII, 327.

lopoulos also saw cells which corresponded with epithelioid cells; while he noted typical Langerhans' and epithelioid cells, especially in liver lesions, he missed the central caseation as it occurs in typical tubercle, which does not quite correspond with the central necrobiosis observed by him in the pseudo lesions. Apostolopoulos studied lesions produced by Pfeiffer's bacillus, but Kirch's examinations were made on pseudo-tuberculous lesions due to the Bacillus paratyphosus B., and are therefore not comparable to the former.

The second type of pseudo-tuberculosis which we met was due to the *Bacillus abortus Bang*, and was first described in this country, in its causal relation in the affection under discussion, by Schröder & Cotton.[1] Our findings are in all respects so entirely in agreement with those of these authors that we reproduce a portion of their description in the following:

"The bacillus of abortion is a non-acid-fast bacillus, with rounded ends, about the size of the tubercle bacillus of the bovine type. On cover glass smears from cultures, stained with Löffler's methylene-blue, the individual bacilli appear very minute and somewhat separated from each other; stained with Stirling's or with anilin gentian violet, they appear to be larger and to lie more closely together; the bacillus is Gram-negative.

"Guinea-pigs become infected through inoculation with or by the ingestion of pure cultures or of naturally infected milk; they show no well-marked lesions until after the passage of six weeks or more. The gross anatomical lesions are an extreme enlargement and edema of the lymph glands generally; the appearance of small glistening nodules in the lungs, which seem to be caused by the enlargement of minute lymph glands that are ordinarily too small to be visible; the conversion of the minute nodules in the lungs into larger necrotic areas; an enormous enlargement of the spleen, often to thirty or forty times its normal volume, an irregular thickening of the capsule of the spleen through which its surface becomes marked with white areas varying in size from mere points to several centimeters in diameter; an enlargement and degeneration of the liver, which organ becomes thickly beset on surface and section with irregular, pale-yellow or dirty-white areas that seem to be due to an enormous proliferation of connective tissue and a consequent crowding out and obliteration of the liver-cells proper; a diffuse parenchymatous nephritis that reaches stages in which dense, fibrous nodules are formed in the cortex of the kidneys; and, in the male guinea-pig, a degeneration of the testicles, commonly beginning in the epididymis and often resulting in the conversion of one or both testicles into structureless cysts filled with creamy pus.

[1] E. C. Schröder & W. C. Cotton; 28th Annual Report; Bureau of Animal Industry for 1911. Washington, 1913, p. 139.

"The milk of a tuberculous cow, which contained both tubercle bacilli and the Bacillus abortus, caused both tuberculosis and the pseudo disease in the guinea-pigs infected with it, showing that the two diseases can live in harmony in one animal body; in fact, each seemed to increase the pernicious potency of the other."

A third type of spontaneous disease in our guinea-pigs, which seemed to be more prevalent than the two types just mentioned, presented little or no macroscopical resemblance to tubercle, and its possible relation to our unexpected results did not suggest itself for some time, the lesions being described in our earlier autopsy-protocols as pneumonic. We do not find this type mentioned in literature at all.

The lesions had the appearance of pneumonic consolidations or infiltrations; they were found in the form of indurated lung areas, usually in the tip of an apex, often singly and very small, one or two millimeters in diameter. In some cases they formed narrow bands along the borders of the upper lobes, and at times the adjacent borders of the other lobes, or the tissues immediately underneath were involved, being affected evidently by contact. There were, however, also scattered or diffused localizations, apparently of more recent date, and it is particularly these that had often been interpreted by us as pneumonic. The condensation of the tissue was then less marked, and its color, which was dark in the typical lesions and closely resembled that of the heart-muscle, was lighter, as it is seen in pneumonia; the foci were then not defined so sharply.

Histologically the sectioned tissue of the more recent lesions were characterized mainly by an infiltration with round-cells; but giant- and epithelioid cells were found in some of them. Older lesions showed more organization with connective and fibrous tissue, presenting the picture of a productive inflammation. The antiformin residue contained no tubercle bacilli.

The histological findings were often confusing, especially at the earlier periods, when we first examined these lesions; this was not only our experience, but that of other skilled pathologists also. Professor Zeit, of Northwestern University Medical School, to whom we submitted specimens for examination, wrote concerning his findings that he could make no other histological diagnosis of the pulmonary lesions than "*lymphangitis tubercu-*

losa perivascularis et peribronchialis," and he added that Professor Kendall agreed with him in this diagnosis.

The cultures made from seared tissues, and from heart-blood taken during life under chloroform, and also immediately after death, always under aseptic precautions, showed a small, Gram-negative coccus resembling that of pneumonia.

Of the various other forms of pseudo-tuberculosis which may be found in guinea-pigs, although they did not come to our notice, mention may be made briefly of one which was described by McCoy & Chapin,[8] who isolated an organism from animals dead of a plague-like disease of rodents, which causes lesions, the subacute or chronic types of which, in guinea-pigs, bear a very strong resemblance to those of tuberculosis. So marked is the similarity that the authors usually stain smears for acid-fast bacilli.

Neisser[9] and Eckersdorff[10] describe the results of their studies made in almost one hundred guinea-pigs affected with pseudo-tuberculosis, the causative virus of which was identified as belonging to the paratyphoid group. In about one-third of the animals the lesions were similar to those of tuberculosis. The typical yellowish-white foci ranged in size from that of a pin-head to that of a pea; they were found almost always in spleen, liver, mesenteric glands and, exceptionally, in the pancreas. Occasionally there were adhesions between the organs; sometimes catarrhal and diffuse swelling of the intestinal mucosa. Kirch cites Dieterlen,[11] who described two cases occurring in the animal stock of the Imperial Health Bureau. Grayish-yellow, pin-head pseudo-tuberculous foci were found in the spleen, evidently due to spontaneous infection with a bacillus which was identified as Bacillus paratyphosus B.; Kirch shows by the results of his own studies that pseudo-tuberculosis in guinea-pigs is often caused by this microörganism. He describes a case which he studied in a guinea-pig which had not been

[8] G. W. McCoy & Ch. W. Chapin; Public Health Bull. No. 53; Washington, 1912; also Journ. of Infect. Dis.; 1912, X, p. 61; and elsewhere.

[9] M. Neisser; Centrlbl. f. Bakt. Ref. Beiheft. 1906, XXXVIII, 99.

[10] Eckersdorff; Arb. a. d. kgl. Inst. f. Experim. Ther. z. Frankfurt a.M.; 1908, Hft. 4, S. 66; cf. E. Kirch, *loc. cit.*

[11] Dieterlen; Arb. a. d. kais. Ges.-Amte.; XXX, Hft. 2; cf. Kirch, *loc. cit.*

treated in any manner and had evidently been infected spontaneously.

On account of the marasmus induced by an associated infection, which we observed so frequently, it may be of interest to cite an older case, reported by Bettencourt,[12] in a lot of guinea-pigs inoculated with material from hypertrophied tonsils and adenoids; among them was an animal that had died twelve days after infection. On the day of infection its weight had been 420 grams; after death, twelve days later, it weighed only 208 grams. Nodules were found in the peritoneum, spleen, liver; the mesentery was studded with them. No Koch's bacilli could be found, but a small bacillus which was identified with Preisz' bacillus of pseudo-tuberculosis. The reviewer adds: "This observation proves the necessity of confirming the autopsy results histologically and by culture experiments, so as to avoid attributing pathological changes to the Koch's bacillus, for which it is not responsible and which are not due to its action."

* * * * *

In the light of these observations, including our own, there can be little doubt that errors have entered into experimental studies in tuberculosis upon guinea-pigs, which were not recognized, and that some of the contradictory findings by different observers, and again by the same observers at different times, may have been due, at least in part if not wholly, to the fact that pseudo lesions were erroneously taken for genuine tubercles. This appears to be the more probable when it is taken into consideration that smears and sections are not made, as a rule, unless the tuberculous nature of the lesions is manifestly doubtful or improbable, a course which we have followed ourselves in the past, and which is still adhered to by other investigators, as far as we know from personal observation and from published experiments. How easy it would then be to err will appear from a description of a single autopsy made in our laboratory 250 days after infection.

GUINEA-PIG No. 418. Weight 400 grams; infected Nov. 12, 1912, with 0.01 mgr. of a human strain of tubercle bacilli, after 24 hours' incubation at 37° C. with four drops of active

[12] A. BETTENCOURT; Archivos de Medicina; 1897, October. Abst. Centrlbl. f. Bakt.; 1898, XXIV, 84.

immune serum taken from a child four years old, 12 months after vaccination. The serum showed complete complement-fixation with all antigens, and the bacilli had undergone marked morphological changes, as shown under the microscope, before they were used for infection. After a loss of 60 grams, during the first two days after infection, the animal held its weight for a week and then recovered its former weight, gaining thereafter until it weighed 560 grams when it was killed on the 250th day after infection.

At the infection site, which was the right axilla, we found a scar; there were two lymph glands in the axilla, about 3 and 5 mm. in diameter, both hard to the touch. On cutting through it, one of them showed slight caseation, the other cut like cartilage; the smear from the first gland showed a few acid-fast fragments and granules; in the antiformin residue from the second gland several well-stained rods were found, and also a few doubtful fragments. No other enlarged glands were found, except one mesenteric lymph node, about 6 mm., from which a whitish, softened material could be pressed out, the smear made being negative as to acid-fast material. The spleen was much enlarged, about 20 by 60 mm., and was full of grayish nodules, ½-1 mm. in diameter, looking exactly like tubercles; the liver presented a similar appearance, only that the organ was not visibly enlarged and the nodules were less in number, several being 3 mm. in diameter. In the omentum six nodules were found, of uniform size, about 2 mm. in diameter; the rest of the abdominal and also the thoracic organs showed nothing abnormal.

We doubt that any one who might have seen the autopsy of this animal would have doubted that he had a tuberculous animal before him. Even though he had made smears from the mesenteric gland, he would not have faltered in his opinion on that account, because tubercle bacilli are not always found in caseous lesions. Sections were made, however, from the spleen, liver and omentum, and no tubercles could be found in them; smears from the antiformin residues were all negative as to tubercle bacilli.

It would be easy to fill pages with similar autopsy findings, including many in which the animals had not been infected by us, or in which our infections were so recent that the formation

of tubercles anywhere could not be considered as due to them. In the present case, we recorded the result as an apparent failure until the section showed that we were justified in claiming the experiment as successful, even though a few acid-fast granules and tubercle bacilli were found in the regional lymph glands, which in itself does not constitute tuberculosis; the less so, as the one gland was undergoing fibrous changes and the other one had become completely fibrous. The animal was in good condition when it was killed, 250 days after infection; the control had died of generalized tuberculosis on the 57th day.

In other cases the appearance of the pseudo lesions would at once cause one to question their genuineness; for instance, when they are few in number, perhaps 5-6 mm. in diameter; even though the frequent necrosis of the center is suggestive of caseation; or when more diffused fibrosis or necrosis is found in patches, in otherwise normal parenchyma. It is often, or even as a rule, possible to judge these lesions correctly by their form and appearance, especially when no infection has been made, or when the short time that has elapsed since infection itself precludes the possibility of any relation to the lesions.

SPONTANEOUS GENUINE TUBERCULOSIS IN GUINEA-PIGS

If the prevalence of pseudo-tuberculosis can interfere very seriously with animal experiments, the same is true to an even greater degree for genuine tuberculosis if this occurs spontaneously or by so-called natural infection, in presumably new and normal animals which have just been received from breeders or dealers. A review of the literature of spontaneous genuine tuberculosis in guinea-pigs has afforded us much interesting information and support.

It may appear strange that observations like ours have not been made more often by other investigators. Although the spontaneous occurrence of pseudo forms of tuberculous disease, and also of the genuine affection, in rodents, was insisted upon occasionally by certain authors, and though even the propriety of employing them for experimentation was questioned sometimes, these small animals were so convenient, and seemed suited

so exceptionally well for experiments with the tubercle bacillus, that no sufficient reason appeared to exist why conditions should be changed. When, however, the more recent studies in immunology demonstrated that even the guinea-pig may develop an immunity to infection, and that even in this animal individual differences exist in the susceptibility to infection and in the evolution and course of tuberculous disease, contradictory observations came to be made with increasing frequency during the attempts to protect these animals against virulent infection, by prophylactic treatment with bacillary products. These contradictory results then called for detailed investigations, and it was found that spontaneous genuine tuberculosis is not as infrequent as was supposed.

Many years ago, Simon [13] had denied the reliability of experiments with animals, because, according to him, tubercle did not occur in rabbits and in other small animals. Virchow,[14] who cites him, adds that he himself has never seen true tubercle in animals.[15]

Villemin [16] called attention to the contradictory views concerning the occurrence of tuberculosis in rodents, some authors claiming that it is frequent, especially in rabbits, while others say that it is rare. Villemin himself saw reason to believe that the disease occurs naturally in rabbits, guinea-pigs and squirrels. He, nevertheless, did not consider this of sufficient importance to exclude these small and convenient animals from employment in his experiments. His work and that of other investigators showed that the views expressed by Simon, Virchow and others were erroneous.

Referring to the assertions that spontaneous tuberculosis is frequent among rodents, Waldenburg held [17] that this view was

[13] JOHN SIMON; General Pathology. London, 1850, p. 168, cf.

[14] RUD. VIRCHOW; Krankhafte Geschwülste. Berlin, II, 1864-5, S. 716.

[15] The reason for this assertion, which appears very strange to us at the present time, lies in Virchow's definition of the true tubercle, and in his refusal to accept anything but the gray miliary tubercle, with its typical structure, as genuine. It is also to be remembered that the bacteriological criterion of a tuberculous lesion was not known at the time when the two works just cited were published; and that "tuberculous" was a pathological and descriptive term, not an etiological one as it is to-day.

[16] J. A. VILLEMIN; Études sur la tuberculose. Paris, 1868, p. 517.

[17] L. WALDENBURG; Die Tuberculose. Berlin, 1869, S. 393.

explained by the fact that every caseous lesion was considered to be tuberculous. In agreement with Virchow, his criterion of what constitutes tubercle was the gray miliary tubercle and, while he frequently observed caseous nodules in his rabbits and guinea-pigs, he said that he never found miliary tubercles, and therefore he denied the supposed or alleged frequent occurrence of tuberculosis in these animals. This opinion the author believed to be supported by the results of Villemin, who kept inoculated and normal animals in the same cage and under like conditions, finding the former affected, the latter not.

In citing the alleged fact that spontaneous genuine tuberculosis either does not occur or that it is extremely infrequent in guinea-pigs, most writers content themselves with quoting Koch's observation, found in his classical monograph on the etiology of tuberculosis,[18] to the effect that among hundreds of recently bought guinea-pigs, which were autopsied in the course of other experiments, he had never once found a tuberculous animal. Spontaneous tuberculosis always occurred in isolated instances and never earlier than three or four months after the animals had been placed in the same room with those that had been infected with tuberculosis. In animals with spontaneous tuberculosis, of which he gives nine autopsy-records in guinea-pigs, and six in rabbits, the bronchial glands were, without exception, found greatly enlarged and suppurating; in most cases the lungs contained a cheesy focus with far-advanced central destruction, so that repeatedly true cavity-formation had occurred, just as in human phthisis. The tubercles in the abdominal cavity were developed far less than those in the lungs. The swelling of the bronchial glands and the commencement of the process in the respiratory organs do not admit any doubt, in Koch's opinion, that spontaneous tuberculosis in these animals is an inhalation-tuberculosis, originating from very few tubercle bacilli or possibly from a single one, and for this reason taking a very slow course. The important point is that, in Koch's opinion, spontaneous tuberculosis is not frequent in guinea-pigs and does not interfere seriously with tuberculosis-experiments.

In the second edition of their textbook, Hérard, Cornil &

[18] ROBERT KOCH; *loc. cit.* (p. 62).

Hanot [19] are of the opinion that spontaneous tuberculosis in guinea-pigs practically does not exist. Straus [20] also says that the guinea-pig and the rabbit, the former of which is very sensitive to inoculation, are never, so to speak, affected with spontaneous tuberculosis. He adds that a spontaneous infection may occur exceptionally in laboratories in which tuberculosis-researches are carried out. Baumgarten [21] does not even admit the cases described by Koch as having been due to contact or "natural" infection, but attributes them to wound-infections, or perhaps to heredity. In over ten years he has not seen a single case of spontaneous inhalation-tuberculosis in his experiment animals, although the conditions for its acquirement were favorable. In a recent edition of his text-book [22] this author says that he has often placed healthy guinea-pigs with those which he had infected in the eye, and that the healthy animals industriously lick the sore eyes of the infected ones; yet he has never seen an ingestion- or any other form of tuberculosis develop in this manner.

Gärtner [23] believes spontaneous tuberculosis to be rare in rodents, and positive reports to be quite exceptional. Among 181 guinea-pigs, which he employed for his investigations, the disease developed spontaneously in only four, that is, in 2.2 per cent.

Of 59 female rabbits which Gärtner kept together with bucks that had been infected in the testicles, only two died of pulmonary tuberculosis in the course of twenty months, in spite of the fact that several of the bucks had open genital ulcers. Of 65 female guinea-pigs, which he kept with male animals infected in like manner, 4 died of tuberculosis in the course of twenty-four months. In two of them the lungs were the primary seat of localization; in the other two this could not be determined. The total frequency of 2.2 per cent of spontaneous tuberculosis in his guinea-pigs was ascertained by careful au-

[19] HÉRARD, CORNIL & HANOT; La Phthisie pulmonaire. Paris, 1888, p. 272.

[20] I. STRAUS; La Tuberculose et son bacille. Paris, 1895, 372.

[21] P. BAUMGARTEN; Lehrbuch d. pathol. Mykologie. Braunschweig, 1890, 614.

[22] P. v. BAUMGARTEN; Lehrb. d. pathogenen Mikroorganismen. Leipzig, 1911, 699.

[23] A. GÄRTNER; Zeitschr. f. Hyg., etc. 1893, XIII, 101.

topsy of all animals that died or were killed, because the author desired to determine the importance of this source of error and to eliminate it. His animals came from various parts of Germany and Austria, and he believes that the frequency found by him constitutes the average.

Leray [24] says that in rabbits and guinea-pigs spontaneous tuberculosis seems to be quite exceptional, in spite of the great susceptibility of the latter animals for tuberculosis-infection. Orth [25] concludes from his experience that the danger of spontaneous infection in guinea-pigs, with tubercle bacilli, is very slight. Hundreds of guinea-pigs live in his institute for years without showing a trace of tuberculosis.

In the study of the lymph gland system, in which Weleminsky [26] unfolds his views concerning the importance of the bronchial lymph nodes for the entire lymphatic apparatus, he relates an experiment in which two guinea-pigs were infected subcutaneously in the inguinal region. On the death of both animals, four weeks after infection, it was found that the regional lymph glands were involved only slightly, but that much older lesions were present, in the upper air passages in one, and in the digestive tract in the other animal. According to a personal communication, Weleminsky found the mesenteric, submental and submaxillary glands enlarged in those cases of spontaneous tuberculosis in guinea-pigs which he had an opportunity to observe; and the infection progressed very slowly. While the spontaneous disease occurred frequently in the old laboratory of the Hygienic Institute in Prague, where the animals were crowded in a dark room, he does not remember having seen a single case since moving into the new laboratory in 1908.

Calmette [27] states that, while the guinea-pig is the most susceptible of all mammalia to experimental infection with tubercle bacilli, it presents the curious peculiarity that it hardly ever acquires tuberculosis spontaneously in breeding establishments.

Liebermeister [28] claims never to have observed spontaneous

[24] Leray; Thèse dé Paris; 1897.

[25] Joh. Orth; Virch. Arch. 1907, CXC, Beiheft, 13.

[26] Fr. Weleminsky; Berl. klin. Wochenschr. 1905, XLII, 1010.

[27] Calmette, Guérin & Breton; Ann. d. l'Instit. Pasteur; 1907, XXI, 401.

[28] G. Liebermeister; Virch. Arch. 1909, CXCVII, 332.

tuberculosis in guinea-pigs, although he paid particular attention to it. It must, however, be added, as Kahn [29] points out, that some of Liebermeister's results certainly suggest spontaneous disease; for instance, his case No. 24, where the guinea-pig had died six days after infection with the patient's blood, and was found to have several caseous foci. Kahn insists that six days is too short a time for caseation to have occurred from the artificial infection. He cites several other instances in Liebermeister's report, which can be explained only by assuming the occurrence of a spontaneous infection.

Although Römer [30] reports some experiments in which three guinea-pigs were found to have spontaneous tuberculosis of the cervical lymph glands, he holds [31] that spontaneous disease is so rare in these animals that its frequency in the stock of the Hygienic Laboratory in Marburg is not over one-half per cent.

We have stated that Simon and Virchow denied the value of experiments in small laboratory animals, because these were not subject to tuberculosis. Other authors objected to their use from the opposite viewpoint, and claimed that the results of experiments upon small rodents, especially guinea-pigs and rabbits, were illusory for the very reason that they acquired the disease spontaneously. Ruge,[32] for instance, relates that Köster, of Würzburg, had reported the occurrence of spontaneous tuberculosis in guinea-pigs, and he himself describes "primary or idiopathic tuberculosis of spontaneous origin" in four guinea-pigs and in one rabbit, bought in Berlin. The lesions were found principally in the lungs, less in the pleuræ and the abdominal organs. Ruge concludes from his experience that "the usually assumed integrity of guinea-pigs with respect to tuberculosis is no longer tenable, and henceforth inoculation-tuberculosis in these animals cannot be taken as a product completely foreign to the guinea-pig character." In a paper read before the Berlin Physiological Society, Bernhardt [33] consid-

[29] Ed. Kahn; Beitr. z. Klin. d. Tuberkulose. 1913, XXVIII, 283 (328).

[30] P. H. Römer; Beitr. z. Klin. d. Tuberkulose. 1909, XIII, 1.

[31] P. H. Römer & K. Joseph; Beitr. z. Klin. d. Tuberkulose. 1910, XVII, 287.

[32] C. A. Ruge; Inaug. Diss. Berlin; 1869.

[33] M. Bernhardt; Centrlbl. f. d. med. Wissensch., 1870, VIII, 276, and Berl. klin. Wochenschr., 1870, VII, 366.

ered the spontaneous occurrence of tuberculosis in guinea-pigs as indubitable, independently of experimental manipulations; in view of the cases which he had observed, he questioned whether these animals were suitable subjects for experimentation. In his well-known article on scrofulosis and tuberculosis, Arloing [34] refers to the "extreme" sensitiveness of the guinea-pig organism to the virus of tuberculosis. Professor Heller, of Kiel, is said by Gärtner [35] to have lost his entire supply of rabbits and guinea-pigs from tuberculosis, when he exposed the normal animals to infection by keeping them in the same stable with the infected animals.

According to Wassermann,[36] it is a well-known fact that, in laboratories in which many tuberculosis-experiments are undertaken, the distribution of the tubercle bacillus, and with it the possibility of its unintended transmission in animal experiments, is quite considerable; also that in the animal stables of these laboratories a not inconsiderable proportion of the highly susceptible guinea-pigs perishes of spontaneous tuberculosis. While some years ago Römer stated, as has been said, that spontaneous tuberculosis in guinea-pigs is of very rare occurrence, and that usually it can be recognized easily, he expressed himself more recently [37] as being convinced that many authors have overlooked the possibility of such a contingency in the interpretation of their results. Although spontaneous tuberculosis is infrequent in guinea-pigs, it certainly does occur and may even be observed in epidemics. This conclusion was probably based upon an experience in the Hygienic Institute, in Marburg, upon which Feyerabend [38] reported recently. This author described certain observations in three lots of guinea-pigs received from the same dealer, in which 19 out of 150 animals were found to have spontaneous tuberculosis. The infection was of the bovine type and was traced to a goat which had been kept in the same pen with the pigs, and with the milk of which the latter had been fed. The goat had recently been coughing and, on being killed, was found to have several

[34] S. Arloing; Rev. d. Méd. 1887, VII, 97.
[35] A. Gärtner, *loc. cit.* (p. 325).
[36] A. Wassermann; Zeitschr. f. Hyg., etc. 1894, XVII, 343.
[37] P. H. Römer; Münch. med. Wochenschr. 1913, LX, 673.
[38] G. Feyerabend; Beitr. z. Klin. d. Tuberkulose. 1913, XXIX, 1.

large cavities filled with pus in the lungs; the udders were studded with nodules. Albahary [39] describes an experiment on three guinea-pigs which received 0.5 cc. of old tuberculin every three days. One of the animals died after six days and was found to be tuberculous. According to Eyre,[40] guinea-pigs are often naturally infected with the bacillus of tuberculosis, and he believes it to be a wise precaution to test the animals as soon as they reach the laboratory by injecting 0.5 cc. of Koch's old tuberculin which causes death in the tuberculous animals in forty-eight hours.

Klopstock & Seligmann [41] believe that "stable-infection" may at times become of importance in animals which are kept for a long time; and Möwes [42] speaks of the possibility of a spontaneous exogenic infection in guinea-pigs.

In referring to the generally acknowledged fact that guinea-pigs are more susceptible to artificial infection with the tubercle bacillus than all other animals, v. Behring [43] also points out that experiments in small animals like these are far more difficult than those in larger animals, and that the results do not admit of direct conclusions or deductions as regards conditions in larger animals and in man. Moreover, it happens that the most interesting guinea-pigs and rabbits are lost, often after prolonged and arduous observation, in consequence of an accidental stable infection or through some other mishap, and that important experiments are seriously disturbed in this manner. For these reasons, v. Behring has, for some years, used the small laboratory animals only for preliminary experiments, but employs larger animals for all important researches in immunization against tuberculosis.

In view of the many positive reports which have been cited, it cannot be denied that spontaneous tuberculosis, independent of artificial infection, may and does occur in guinea-pigs and

[39] J. M. Albahary; Münch. med. Wochenschr. 1914, LXI, 1385.

[40] J. W. H. Eyre; The Elements of Bacteriologic Technic. Philadelphia and London, 2nd ed., 1913, 339.

[41] F. Klopstock & W. Seligmann; Zeitschr. f. Hygiene. 1914, LXXVI, 77.

[42] C. Möwes; Veröffent. d. Rob. Koch. Stiftung. Berlin, 1914, Hft. 5, S. 44.

[43] E. v. Behring; Beitr. z. experim. Ther. Marburg, Hft. 5, 1902, S. xvii, xviii.

that, in these animals as in all others and in man, it is simply a question of exposure to infection. In support of this view, the experiments of Bartel & Spieler [44] are of interest.

These authors report experiments upon guinea-pigs which they exposed to infection in rooms occupied by consumptives. The exposure was not forced in any way, that is, the animals were not deliberately coughed at or spat at by the patients, as had been the procedure in several older experiments reported in literature, but they were simply kept in the same rooms as the patients, being exposed very much in the same manner as small children would be. The exposure lasted from four hours to several days, after which the animals were returned to the Institute. Some of them developed marasmus without tuberculosis; others became tuberculous; still others were not anatomically tuberculous, but their swollen lymph glands were found to contain tubercle bacilli on histological and also on bacteriological examination, that is, in culture and inoculation experiments.

Wolff-Eisner [45] kept a number of guinea-pigs for two weeks in the residence of a consumptive patient, and others in a ward in the Krankenhaus Friedrichshain, in Berlin. After six months, and up to nine months after the exposure, the animals continued in good health and increased in weight. Then they lost, at first slowly and later more rapidly, and finally they died. Autopsy showed enormous swelling of the deep cervical lymph nodes, from which the tuberculous process had extended to the pleuræ, lungs, spleen, liver. The intestines and mesenteric glands were free from tuberculous changes.

A number of years ago, an unintentional experiment of like character was made in the Winyah Sanatorium, which was never published. The little daughter of one of the patients asked for some guinea-pigs as pets, and was permitted to keep two animals in her room. Her father was a rather difficult patient to control, and probably was not very careful with his expectoration; at any rate, the guinea-pigs died of tuberculosis in course of time.

Quite recently Dr. Chapin,[46] the superintendent of health at

[44] J. Bartel & F. Spieler; Wien. klin. Woch. 1905, XVIII, 218.
[45] A. Wolff-Eisner; Berl. klin. Woch. 1908, XLV, 2314.
[46] Ch. V. Chapin; Journ. Amer. Med. Assoc. 1914, LXII, 423 (430).

Providence, Rhode Island, reported on a further experiment which belongs here and which was made in a house in Providence, occupied by a careless consumptive patient. Here two sets of guinea-pigs were exposed in cages; one set being fed by the patient, and the other excluded from every form of direct contact with him. Most of the animals in both sets developed tuberculosis. The animals in the locked cage covered with wire-cloth were, in Chapin's opinion, infected by mouth-spray, because the patient often held his face right in front of the box and talked to the animals.

Finally, Chaussée [47] reports on an experiment in which a cage containing thirteen guinea-pigs was kept, during the summer months, in the consumptive-ward of the *Hôpital Boucicaut*, in Paris. Not one of these animals contracted tuberculosis, although the cage was close by the bed of a consumptive patient who coughed "enormously" and brought up immense numbers of tubercle bacilli with his sputum. A similar experiment, however, undertaken in mid-winter, when the doors were kept shut and the windows closed almost entirely, at night, resulted in the infection of 10 out of 16 guinea-pigs. Out of a total of 76 guinea-pigs exposed in various ways to "natural" infection, 30 became infected, and autopsy showed that in every instance the infection had been acquired by inhalation.

In our autopsies of animals which had acquired a spontaneous genuine infection with tubercle bacilli, we had an opportunity of studying the localization of the lesions, with reference to the probable mode by which they had acquired their infection, and also the subsequent extension of the disease. In a lot of 400 guinea-pigs, received from a breeder who sold out his stock because of failing health (on account of which he moved to California), there were apparently but few animals that had not acquired a spontaneous infection. Several died soon after arrival, and a number of them succumbed during the first two weeks after receiving one or two small doses of our vaccine; among them we found instances of pulmonary tuberculosis without enlarged lymph glands in the chest or anywhere else, and only when the lesions were extensive, more particularly after caseation had occurred, did we find the bronchial glands enlarged

[47] CHAUSSÉE; Ann. d. l'Inst. Pasteur; 1914, XXVIII. Cf. Brit. Med. Journ.; 1915, I, 605.

and tuberculous. In other animals we found no macroscopical lesions, but noted characteristic focal reactions in one or both upper lobes; sections made of the reacting tissues contained typical tubercles. It was also worthy of note that the old lesions were usually found in the upper lobes, in one case with a well-formed lung cavity; the disease had apparently not extended beyond this upper lobe, although the hilus-glands were enlarged but not yet caseous. In cases in which secondary extension to the abdomen had taken place, the mesenteric glands seemed to be the first abdominal localization, some animals showing nothing more than their involvement.

In other instances the thoracic organs were entirely free from all evidence of tubercle, but the abdominal organs were involved more or less; we considered these cases, therefore, as instances of infection by ingestion. Among them were animals which had died after a dose of vaccine, apparently without tubercles anywhere, the interesting feature of their autopsy findings being that some part of the digestive tract, most often a short loop of the intestine, and in one case the pyloric end of the stomach, showed intense congestion. In these localities of focal reactions, we then found tubercle bacilli in the antiformin residue, and histological tubercles in the sections, as we had seen them in lung areas that showed similar reactions, at a time when the bronchial lymph glands were still free. Tubercles distributed along the course of the blood-vessels of the mesentery were also found frequently at periods before the spleen and the liver showed evidences of disease. Extension to the chest, as a rule, was associated with caseation of the retroperitoneal glands. In such cases the hilus- and bronchial glands were always enlarged and often caseous, and the lung lesions appeared to be of more recent date, or focal reactions were noted near the hilus and in the lower lobes without macroscopic lung lesions.

In both modes of infection we found occasionally enlarged caseous and non-caseous cervical lymph nodes.

Apart from this lot of 400 guinea-pigs, we found between fifty and sixty other instances of genuine spontaneous tuberculosis during the last three years. The slow course of these spontaneous infections was a marked feature and presented a decided contrast to that following upon artificial infections.

With other observers we are of the opinion that the large number of tubercle bacilli, introduced in artificial infection at one time directly into the tissues, explains the difference, but it is probable that the possible attenuation of the virus, since its discharge from its former host, may likewise contribute in bringing about the comparatively benign course of a spontaneously acquired infection, as is suggested by the fact that the course of artificial infection may be similarly mild, in cases in which it is made with a culture of slight virulence.

In his paper on the heredity of tuberculosis, to which we have already referred, Professor Gärtner states that tuberculosis, due to natural or spontaneous infection, depends upon the opportunities for it, and also upon the natural provisions for defense, which the organism possesses. Rabbits and guinea-pigs are very susceptible to tuberculosis, yet they do not appear to acquire the disease frequently by inhalation. Gärtner attributes this, in part, to the fact that these animals constantly breathe through the nose and that the inhaled bacilli are filtered out better in their labyrinth-like nasal passages than is the case in man. Moreover, rabbits and guinea-pigs do not cough and, therefore, do not expectorate tubercle bacilli. With the feces and urine the bacilli are eliminated only if tuberculous ulcerations are present in the intestinal tract or in the genito-urinary apparatus. Consequently there are only few or no tubercle bacilli in the environment of rodents, which fact renders the infection of "new" animals infrequent.

Gärtner's argument is of interest and may serve to account for the actual fact that guinea-pigs, which are so exceedingly susceptible to artificial infection, do not often, relatively speaking, succumb to natural infection, the reason being that they are exposed to it comparatively rarely; but their first provisions of defense which, according to Gärtner, serve to protect them against the disease if they inhale tubercle bacilli, are probably of relatively slight value, since it has been found that guinea-pigs acquire a natural infection and, following it, a chronic tuberculosis, almost certainly after such an inhalation.

Certain individual differences exist, however, even in guinea-pigs of the same age and weight and living under like conditions, in their response to like infections with tubercle bacilli.

Max Wolff [48] pointed this out in the discussion of Orth's report on the results of infections made upon animals immunized by Friedmann, and we ourselves have made like observations upon normal animals. If spontaneous tuberculosis is relatively rare among guinea-pigs, the justifiable inference is that, under normal conditions, the opportunity for infection is not great.

From all these considerations, the conclusion must be drawn that guinea-pigs are not only highly susceptible to artificial infection with virulent tubercle bacilli, but that they are also subject to ordinary, epidemiological infection if they are exposed to it, and that they respond to it in like manner as do other susceptible animals, and humans, in accordance with the massiveness and virulence of the infection. Our observations in the lot of 400 guinea-pigs, which had been exposed to natural infection, have shown that the frequency of tuberculosis, acquired in this manner by guinea-pigs, does not differ from that in other animals or in human subjects. If it is considered that the guinea-pig is a very small animal and that its organs are very small, it is easily understood that the duration of the disease in them is comparatively shorter, because the vital organs become involved sooner and because they become unable to sustain life at an earlier period than is the case in large animals.

The evidence, which we have presented in the preceding pages, establishes the justice of our contention that the results of tuberculosis-studies in guinea-pigs and, to a lesser degree, in rabbits cannot be accepted as conclusive, unless all lesions that are found on autopsy are examined histologically and bacteriologically.

THE VALUE OF DIAGNOSTIC INOCULATIONS IN GUINEA-PIGS

It is not only in experimental studies that the liability of guinea-pigs to genuine spontaneous, and also to pseudo-tuberculosis, is deserving of attention; this possible source of error is of even greater practical importance in their employment for diagnostic inoculations. For many years the guinea

[48] Max Wolff; Berl. klin. Woch. 1909, XLIV, 1057.

pig has served for diagnostic experiments, in order to determine or exclude the tuberculous nature of suspected lesions. If, after infection with a certain tissue or secretion, the animal remains free from manifest tuberculosis, this fact is accepted as a positive proof that the injected material is not of tuberculous origin; on the other hand, if the animal shows nodular or caseous lesions on autopsy, a diagnosis of tuberculosis is made unhesitatingly on the strength of the macroscopic findings. When it is desired to settle a question of clinical diagnosis experimentally in the shortest possible time, as is almost always the case, the animals are killed as early as twenty or thirty days after infection, and attempts have been made to shorten even this period of diagnostic experimentation. In the light of our experience, the results of such experiments may be accepted as positive only in case the infection has followed the typical course of extension, first to the regional, and later to distant lymph glands and to distant organs, and if the bacteriological and histological examinations give unquestionable confirmation; whereas negative findings are of no value in any case, even if the animals have lived many months. We base this view upon the fact that, in experimental infection, the evolution of tubercle differs according to the number of living tubercle bacilli which have been introduced, according to the degree of their virulence, and according to the individual resistance of the animal; the latter factor is to be emphasized especially, because marked differences occur even when all conditions of the infection have been equal. When, however, we make infections with material in which the factors of numbers and virulence are unknown, we must expect failures even though the material used has a relation to a genuine tuberculous lesion. In a given diagnostic experiment the material may not contain enough virulent tubercle bacilli to cause an infection, or it may not give rise to macroscopical lesions or histological changes during weeks and months, on account of the small number and the low virulence of the bacilli, and later yet cause the development of typical tuberculous lesions. This fact is sufficient to disqualify attempts of drawing inferences from the results of autopsies which are made a few weeks or months after diagnostic infections, unless the outcome is unquestionably positive; yet, as a matter of fact, such experiments are made more often in order to exclude than

to establish the diagnosis of tuberculosis. In either case the information is, as a rule, of little or no practical value unless it can be obtained with sufficient promptness.

Believing that the importance of the subject justifies us in the concise description of observations which, in our opinion, support us in our viewpoint, we call attention to the several groups of experiments which follow:

GROUP I

Series of six guinea-pigs, all killed 21 days after subcutaneous infection with 0.2 cc. of tubercle bacillus emulsion; this dose equaled 0.01 mgr. of tubercle bacilli.

GUINEA-PIG No. 1. Infection site negative; regional lymph glands negative; internal organs, pseudo-tuberculosis of lungs; sections show no tubercles. *No tuberculosis.*

GUINEA-PIG No. 2. Infection site negative; regional lymph glands enlarged, 3 mm., soft; antiformin residue smear shows tubercle bacilli; internal organs negative. *Tubercle bacilli in regional lymph glands.*

GUINEA-PIG No. 3. Infection site: nodules, 2 mm., tubercle bacilli in smears; regional lymph glands enlarged, central caseation, tubercle bacilli in smears, histological tubercles in sections; internal organs, few miliaries in spleen, tubercle bacilli in antiformin residue, sections show many tubercles. One mesenteric gland enlarged, 3 mm., soft, tubercle bacilli in antiformin residue; other organs normal. *Beginning tuberculosis.*

GUINEA-PIG No. 4. Infection site negative. Regional lymph glands negative. Internal organs negative. Sections from spleen, no tubercles. *No tuberculosis.*

GUINEA-PIG No. 5. Infection site negative. Regional lymph glands, 2 mm., soft; antiformin residue negative. Internal organs, spleen slightly enlarged, one suspicious nodule, 1 mm. Sections show no tubercles. No tuberculosis. *Pseudo-tuberculosis of spleen.*

GUINEA-PIG No. 6. Infection site negative. One regional lymph gland enlarged, 2 mm.; section shows tubercles. Internal organs show pseudo-tuberculosis of lung. Sections from lung, spleen, liver show no tubercles. *Lymph gland tuberculosis and pseudo-tuberculosis of lung.*

GROUP II

Four guinea-pigs, killed 42 days after subcutaneous infection with 0.2 cc. of tubercle bacillus emulsion, the dose equaling 0.01 mgr. of tubercle bacilli.

GUINEA-PIG No. 1. Infection site, 5 mm. caseous; tubercle bacilli in smears. Regional lymph glands enlarged, caseous; tubercle bacilli in smears. Internal organs: mesenteric glands enlarged, hard, 5 mm.; antiformin residue, few tubercle bacilli. Spleen, few miliaries, with tubercle bacilli in antiformin residue; sections show many tubercles. Liver, several

nodules, 1-2 mm., hard; antiformin residue negative; sections show no tubercles. Other organs normal. *Genuine tuberculosis. Pseudo-tuberculosis of liver.*

GUINEA-PIG No. 2. Infection site negative. Two regional lymph glands enlarged, 2-5 mm., soft; antiformin residue of one gland negative; section of the other gland shows no tubercles. Internal organs: spleen slightly enlarged, few miliaries; antiformin residue negative; section shows no tubercles. *No tuberculosis. Pseudo-tuberculosis of spleen.*

GUINEA-PIG No. 3. Infection site, 3 mm., caseous; tubercle bacilli in smears. Regional lymph glands enlarged, 2-5 mm., one with central caseation; tubercle bacilli in smears. Internal organs normal. Section of spleen shows no tubercles. *Tuberculosis of regional lymph glands.*

GUINEA-PIG No. 4. Infection site, diffused thickening; no caseation; antiformin residue, many tubercle bacilli. Regional lymph glands negative. Internal organs: liver, one soft necrotic area, 5 mm.; smears, no tubercle bacilli, two hard foci, 3-4 mm., antiformin residue negative, sections show no tubercles. *Tubercle bacilli in infection site. No tuberculosis. Pseudo-tuberculosis of liver.*

GROUP III

Thirteen guinea-pigs killed at successive periods, after infection with tubercle bacilli from a culture of slight virulence.

GUINEA-PIG No. 1. Infected with 0.08 mgr. of tubercle bacilli, subcutaneously in left flank. Killed 68 days later. Infection site negative. Regional lymph glands negative. Omentum contains one gland, 4 mm.; histological examination shows no tubercles. Two miliary nodules on serous covering of left testicle; sections show no tubercles. One poststernal gland, 3 mm., soft; section shows no tubercles. *No tuberculosis.*

GUINEA-PIG No. 2. Infected with 0.01 mgr. of tubercle bacilli; killed 80 days later. Infection site negative. Regional lymph glands negative. Few nodules upon serous surfaces of right and left testicles. Spleen apparently normal. Sections of spleen show few miliary tubercles. *Beginning tuberculosis of spleen.*

GUINEA-PIG No. 3. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 100 days later. Infection site negative. Left inguinal gland enlarged, soft. Section shows few tubercles. All organs normal. Sections from spleen, liver, mesenteric glands, negative. *Tubercles in regional lymph gland.*

GUINEA-PIG No. 4. Infected with 0.08 mgr. of tubercle bacilli, subcutaneously. Died after 128 days. Infection site negative. Regional lymph glands 3 mm., soft; sections show caseating tubercles. Nothing in internal organs. *Tuberculosis of regional lymph glands.*

GUINEA-PIG No. 5. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed after 146 days. Infection site, 7 mm. fibrocaseous; smears show tubercle bacilli. Regional lymph glands caseous. Cervical gland, 15 mm., caseous; smear shows tubercle bacilli. Mesenteric glands enlarged, 5 mm., soft; antiformin residue shows tubercle bacilli. Spleen enlarged; several 2 mm. nodules; antiformin residue negative; section shows

hyperplasia, no typical tubercles. *Lymph gland tuberculosis. Pseudo-tuberculosis of spleen.*

GUINEA-PIG No. 6. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 174 days later. Infection site, 7 mm. fibrous area; antiformin residue shows acid-fast granules. Regional lymph glands, 7 mm. fibrous; antiformin residue shows tubercle bacilli; section shows fibrous tubercles. Mesenteric gland 3 mm., soft; antiformin residue shows few tubercle bacilli; section shows caseous tubercles and fibrosis. *Tuberculosis.*

GUINEA-PIG No. 7. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 174 days later. Infection site, 3 mm., fibrous area; antiformin residue shows tubercle bacilli. Regional lymph glands, 10 mm., hard; section shows tubercles and fibrosis. Retroperitoneal gland, 5 mm., hard; antiformin residue shows tubercle bacilli. Liver, few nodules, 1-2 mm.; section no tubercles. Spleen enlarged 20 by 40 mm., full of 1 mm. nodules; antiformin residue shows tubercle bacilli and granules; section shows fibrous tubercles. Lungs, few 1-2 mm. nodules in both; section shows miliary and caseating tubercles. *Tuberculosis. Pseudo-tuberculosis of liver.*

GUINEA-PIG No. 8. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 188 days later. Infection site negative. Regional lymph gland enlarged, 5 mm.; few tubercles in section. Mesenteric gland, 5 mm.; antiformin residue negative. Liver and lungs full of fibrous areas, 2-5 mm.; antiformin residues negative; sections show no tubercles. Spleen enlarged, full of 1-3 mm. soft nodules; antiformin residue shows tubercle bacilli; section shows breaking-down tubercles. *Tuberculosis and pseudo-tuberculosis of liver.*

GUINEA-PIG No. 9. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 188 days later. Infection site negative. Regional lymph gland, 15 mm., hard; antiformin residue shows tubercle bacilli. Mesenteric gland, 7 mm., soft; antiformin residue shows tubercle bacilli. Spleen, 10 by 30 mm., full of 1-2 mm. soft nodules; antiformin residue shows tubercle bacilli; section shows miliary tubercles. Lungs, many small, hard nodules and larger areas of fibrosis; antiformin residue shows tubercle bacilli; sections show few tubercles. *Tuberculosis.*

GUINEA-PIG No. 10. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 188 days later. Infection site negative. Regional lymph gland enlarged, central caseation; smears show tubercle bacilli. Liver, suspicious granular appearance; section shows miliary tubercles. Spleen enlarged, 10 by 25 mm., no macroscopic tubercles; antiformin residue shows tubercle bacilli. Retroperitoneal gland, 2 mm.; antiformin residue shows few tubercle bacilli. Lungs, few miliaries; antiformin residue shows tubercle bacilli and granules; section shows miliary tubercles. *Tuberculosis.*

GUINEA-PIG No. 11. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 203 days later. Infection site negative. Regional lymph gland, 6 mm., central caseation; smears show tubercle bacilli. Mesenteric gland, 10 mm., hard; antiformin residue shows tubercle bacilli; section shows caseating tubercles. Liver, few 1 mm. nodules; section shows caseating tubercles. Spleen enlarged, 15 by 40 mm., full of 1 mm. hard nodules;

antiformin residue shows tubercle bacilli; section shows caseating tubercles and slight fibrosis. Bronchial gland, 7 mm., hard; antiformin residue shows tubercle bacilli. *Tuberculosis.*

GUINEA-PIG No. 12. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 203 days after infection. Infection site negative. Regional lymph gland, 9-10 mm., central caseation; smear contains tubercle bacilli. Mesenteric gland, 5 mm., soft; antiformin residue contains tubercle bacilli. Retroperitoneal gland, antiformin residue shows tubercle bacilli. Liver full of 1-3 mm. nodules; section shows infiltration and breaking-down tubercles. Spleen enlarged, 20 by 30 mm., same as liver; antiformin residue shows tubercle bacilli and granules. Lungs, several 2 mm. hard nodules; antiformin residue negative; section shows peribronchial and perivascular infiltration and fibrosis; no typical tubercles. *Tuberculosis. Pseudo-tuberculosis of lungs.*

GUINEA-PIG No. 13. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Died 240 days after infection. Infection site negative. Regional lymph glands 3 mm., hard. Mesenteric gland, 5 mm., hard; antiformin residue shows tubercle bacilli. Retroperitoneal lymph gland, tubercle bacilli in antiformin residue. Liver full of 1-2 mm., soft nodules; tubercle bacilli in antiformin residue. Spleen, 20 by 50 mm., full of miliaries. Lungs, few miliaries; antiformin residue shows tubercle bacilli. Right middle lobe pneumonic; section of pneumonic portion shows miliary tubercles. *Tuberculosis.*

DIAGNOSTIC EXPERIMENT WITH SPUTUM

TWO ANIMALS USED

GUINEA-PIG No. 1. Died after 69 days. Infected with antiformin residue of sputum, subcutaneously in the right axilla.

Infection site negative. Regional lymph glands negative. Internal organs normal, except lung which shows small area of pseudo-tuberculosis; antiformin residue negative; section shows peribronchial and perivascular infiltration; no tubercles. *No tuberculosis. Pseudo-tuberculosis of lung.*

GUINEA-PIG No. 2. Infected same as guinea-pig No. 1. Died after 117 days. Infection site caseous nodules, 1 mm.; smear shows tubercle bacilli. Regional lymph gland, 5 and 7 mm., hard; antiformin residue shows tubercle bacilli. Mesenteric gland, enlarged; antiformin residue shows few tubercle bacilli. Liver, 3 foci, 5 mm., hard; antiformin residue negative. Spleen enlarged, few miliary tubercles; antiformin residue contains tubercle bacilli; section shows miliary tubercles, several foci like those in liver; antiformin residue negative; section shows necrosis and fibrosis, no tubercles. Lungs full of fibrous areas, 1-3 mm.; antiformin residue negative; section shows peribronchial and perivascular fibrosis; no tubercles. *Tuberculosis of regional lymph glands and spleen. Pseudo-tuberculosis of liver, spleen, lungs.*

It seems to us that the foregoing experiments bear out the view that the evolution of an infection in guinea-pigs may differ

greatly in individual animals, although the infection has been the same in all respects. Without closer examination, Guinea-pigs No. 2 and 3, of Group III, could easily have passed for normal, while Guinea-pig No. 1 showed no evidence of the infection on the 21st day. This was, however, only a question of virulence, as appears from later autopsies. Guinea-pig No. 4, autopsied after 128 days, could still have passed as normal if the small soft inguinal lymph glands had been overlooked or if they had not been sectioned. The subsequent autopsies, on Guinea-pigs Nos. 5-13, at periods between 146 and 240 days after infection, indicate that the infection of Guinea-pig No. 1, which was autopsied on the 68th day, had at that time not developed sufficiently to cause genuine tuberculous lesions, although the pseudo lesions could easily have been mistaken for genuine ones.

Even more instructive are the two diagnostic experiments with sputum, in which nothing in the form of tubercle was as yet in evidence, on the 69th day, that could have had any relation to our infection, nothing being found at the infection site or in the regional lymph glands, and the small area of infiltration found in one lung showing neither tubercle bacilli nor histological tubercles. Guinea-pig No. 2, like No. 1, had received 0.5 cc. of saline suspension of the antiformin residue of the suspected sputum in which the microscope had shown few acid-fast fragments and granules. This animal died after 117 days, apparently from the effects of pseudo-tuberculosis with beginning miliary tuberculosis in the spleen. By this time a small caseous lesion had developed at the infection site, the regional lymph glands had become enlarged but had not caseated; the infection had extended to the mesenteric glands and the spleen. Had this animal also died earlier, or had it been killed prematurely, a negative result would have been recorded when, in fact, the infection only lacked in sufficient virulence to cause a more rapid evolution, as was the case in the animals of Group II. We can duplicate this diagnostic experiment in a case of suspected tuberculosis of a kidney, in which one animal was killed after 60 days and the other after 120 days from the date of infection; nothing was found in the first, and evident tuberculous lesions, including involvement of the regional lymph glands, were present in the second.

These and other experiments have convinced us that diag-

nostic experiments with suspected tuberculous material cannot be accepted as conclusive at a time when the information sought is most valuable, unless the findings are positive, and that, in order to assure the relation of existing lesions to the infection, this must be shown to have involved the regional lymph glands, and the tuberculous nature of the lesions must be established by positive results in the examination of smears and sections.

More confusion and serious errors are, however, likely to occur through pseudo lesions which may resemble miliary and nodular tuberculous lesions so closely macroscopically that no doubt as to their true nature is apt to be entertained. The liability to error is increased by the early breaking-down of the infection site and by the enlargement or suppuration of the regional lymph glands, an occurrence which we have observed so frequently that its relation to the coexisting pseudo infection is to us as undoubted as it is difficult to find an explanation for it.

Schröder & Cotton are of the opinion that, in genuine tuberculosis coexisting with the type of pseudo-tuberculosis described by them, each seemed to increase the pernicious potency of the other, and we do not doubt that this is often the case. In our experience, however, there appears to be another factor peculiar to the behavior of the infection site which, in some instances, we have found to present an abscess as early as the 10th and 12th days; on the bacteriological examination of these abscesses we have frequently been able to demonstrate the same organism which we found in the lesions of internal organs or in the blood of animals that had not been infected by us, suggesting that the organisms reached the infection site from within, through the medium of the blood. The abscesses may open and discharge, and thereafter they may heal; and we have met with instances, after the early discharge of an abscess, in which the purulent material and the nearby enlarged lymph glands contained no tubercle bacilli, and where the sections from abscess-wall and lymph glands were negative as to the presence of tubercles. It appears as though, in these cases, the injected tubercle bacilli had been discharged with the contents of the abscess.

The objection might be raised, in connection with our experiments, that our tubercle bacillus emulsions were not sterile and

that they conveyed the microörganisms which caused abscess-formation. We have, however, made these observations only in animals which had pseudo lesions; if such a contamination were responsible for our unusual results, it would naturally be expected that the result would be the same in all animals, or at least in most of those that were infected with a certain emulsion at the same time; but this was not the case.

Not all guinea-pigs, in which we found pseudo lesions on autopsy, showed these early changes at the infection site, nor did all of them die soon after infection. Quite the contrary, a considerable number appeared to behave normally or nearly so, and they died or were killed months later. Then it was, of course, impossible to decide whether the pseudo lesions, which were found, were due to infections acquired before or after the time when we ourselves infected the animals with tubercle bacilli. In our opinion, it is probable that the pseudo infection was acquired later in at least a considerable number of the animals, inasmuch as they were constantly exposed to it. In immunized animals, and also in those which had been infected with tubercle bacilli after these had been subjected to the bactericidal action of immune serum, the deaths often occurred many months after infection, and the animals had progressed favorably and had gained steadily in weight up to a short time before death. These animals frequently died within a few weeks after the first loss of weight was noted, suggesting that the unfavorable influence had become active only recently, as would be expected in pseudo-tuberculosis of the Pfeiffer type.

CHAPTER XII

ANIMAL EXPERIMENTS WITH BACTERICIDAL SERA

In our report of 1912 some bactericidal experiments were mentioned as not completed, the animals having been kept for further observation. The animal in Group I which had been infected intraperitoneally with 0.01 mgr. of tubercle bacilli incubated with INACTIVATED immune serum taken from a patient treated with Watery Extract of Tubercle Bacilli, died 99 days after infection. The animal had lost 200 grams since its infection. The autopsy showed infection site and regional lymph glands caseous; generalized tuberculosis of abdominal organs and lungs; smears and sections were positive.

The records of the five animals in Group II are as follows:

GUINEA-PIG No. 23. Infected intraperitoneally with 0.01 mgr. of tubercle bacilli incubated with active immune serum of a patient treated with Watery Extract of Tubercle Bacilli, taken eight years after discharge; weight 560 grams. Died 739 days after infection, of acute pneumonia; weight 700 grams. Aside from the pneumonia, the only lesion found was a scar, 5 mm. in diameter, in the liver; smears from antiformin residue of liver lesion and of pneumonic lung were negative as to tubercle bacilli; smears from cut surface of lung showed diplococci.

GUINEA-PIG No. 24. Infected the same as Guinea-pig No. 23; serum used came from a patient on discharge, after treatment with Watery Extract of Tubercle Bacilli; weight 820 grams. Killed 180 days after infection; weight 770 grams. Autopsy: Infection site and regional lymph glands not found; two hard glistening nodules, about 1 mm., in omentum, and two similar nodules in the liver; no tubercle bacilli in antiformin residue; sections show fibrous structure.

GUINEA-PIG No. 25. Infected same as Guinea-pig No. 23. Serum obtained from a patient after treatment with Watery Extract of Tubercle Bacilli; weight 560 grams. Killed 184 days after infection; weight 725 grams. Autopsy: normal conditions throughout, except one minute hard nodule in mesentery. Section showed fibrous structure.

GUINEA-PIG No. 36. Infected same as Guinea-pig No. 23. Serum taken from a child, five days after vaccination; weight 810 grams. Died 120 days later of acute pneumonia; weight 750 grams. Autopsy, infection site and

regional lymph glands not found; omentum a contracted fibrous mass; several necrotic foci in liver and spleen; no tubercle bacilli found in lesions; sections negative (apparently pseudo-tuberculosis, Pfeiffer type, of liver and spleen).

GUINEA-PIG NO. 37. Infected same as Guinea-pig No. 23. Serum taken from a child eight months after vaccination; weight 490 grams. Died 313 days after infection; animal had gained continuously for five months and had reached a weight of 870 grams; thereafter it lost gradually until death; weight 550 grams. Autopsy: infection site not found; one regional (inguinal) lymph gland caseous; a mesenteric gland enlarged, hard; necrotic areas in liver and spleen; smears and sections all negative (apparently pseudo-tuberculosis, Pfeiffer type, in liver and spleen).

The four control animals for these infections had died of generalized tuberculosis between 32 and 42 days after infection.

Until June, 1914, when we were obliged to relinquish them on account of defective antigens, we continued the examination of sera, before and after vaccination or treatment, for the several antibodies, and we included in these examinations determinations of the alkalinity of the blood. The sera of the cases to be considered in the experimental details which follow, were all examined in this manner, and our records show no material difference from the average results as we gave them in our report of June, 1912, and in previous publications. We desire again to call attention to the uniform increase of the blood alkalinity, which we have observed after the administration of specific remedies in the form of the Watery Extract of Tubercle Bacilli and the Vaccine, and reproduce the summary of our observations in the examinations of 339 vaccinated children, from the report mentioned above.

BLOOD ALKALINITY[1]

	Average	Minimum	Maximum
Preliminary examination	0.85	0.60	1.00
After 1st dose	1.05	0.90	1.20
After 2nd dose	1.20	1.05	1.30
After 3—8 months	1.20	1.10	1.30

[1] The blood alkalinity was estimated by Dare's spectroscopic method, which is based on a normal of 266 milligrams sodic hydrate per 100 cubic centimeters of blood. We have repeatedly controlled the results obtained by Dare's method, with titration-methods and found no material differ-

nodules, 1-2 mm., hard; antiformin residue negative; sections show no tubercles. Other organs normal. *Genuine tuberculosis. Pseudo-tuberculosis of liver.*

GUINEA-PIG No. 2. Infection site negative. Two regional lymph glands enlarged, 2-5 mm., soft; antiformin residue of one gland negative; section of the other gland shows no tubercles. Internal organs: spleen slightly enlarged, few miliaries; antiformin residue negative; section shows no tubercles. *No tuberculosis. Pseudo-tuberculosis of spleen.*

GUINEA-PIG No. 3. Infection site, 3 mm., caseous; tubercle bacilli in smears. Regional lymph glands enlarged, 2-5 mm., one with central caseation; tubercle bacilli in smears. Internal organs normal. Section of spleen shows no tubercles. *Tuberculosis of regional lymph glands.*

GUINEA-PIG No. 4. Infection site, diffused thickening; no caseation; antiformin residue, many tubercle bacilli. Regional lymph glands negative. Internal organs: liver, one soft necrotic area, 5 mm.; smears, no tubercle bacilli, two hard foci, 3-4 mm., antiformin residue negative, sections show no tubercles. *Tubercle bacilli in infection site. No tuberculosis. Pseudo-tuberculosis of liver.*

GROUP III

Thirteen guinea-pigs killed at successive periods, after infection with tubercle bacilli from a culture of slight virulence.

GUINEA-PIG No. 1. Infected with 0.08 mgr. of tubercle bacilli, subcutaneously in left flank. Killed 68 days later. Infection site negative. Regional lymph glands negative. Omentum contains one gland, 4 mm.; histological examination shows no tubercles. Two miliary nodules on serous covering of left testicle; sections show no tubercles. One poststernal gland, 3 mm., soft; section shows no tubercles. *No tuberculosis.*

GUINEA-PIG No. 2. Infected with 0.01 mgr. of tubercle bacilli; killed 80 days later. Infection site negative. Regional lymph glands negative. Few nodules upon serous surfaces of right and left testicles. Spleen apparently normal. Sections of spleen show few miliary tubercles. *Beginning tuberculosis of spleen.*

GUINEA-PIG No. 3. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed 100 days later. Infection site negative. Left inguinal gland enlarged, soft. Section shows few tubercles. All organs normal. Sections from spleen, liver, mesenteric glands, negative. *Tubercles in regional lymph gland.*

GUINEA-PIG No. 4. Infected with 0.08 mgr. of tubercle bacilli, subcutaneously. Died after 128 days. Infection site negative. Regional lymph glands 3 mm., soft; sections show caseating tubercles. Nothing in internal organs. *Tuberculosis of regional lymph glands.*

GUINEA-PIG No. 5. Infected with 0.01 mgr. of tubercle bacilli, subcutaneously. Killed after 146 days. Infection site, 7 mm. fibrocaseous; smears show tubercle bacilli. Regional lymph glands caseous. Cervical gland, 15 mm., caseous; smear shows tubercle bacilli. Mesenteric glands enlarged, 5 mm., soft; antiformin residue shows tubercle bacilli. Spleen enlarged; several 2 mm. nodules; antiformin residue negative; section shows

overcome, the primarily depressed curve of the alkalinity takes an upward tendency and frequently reaches higher degrees than were observed before the infection occurred. A material increase in the blood alkalinity in an infectious disease is considered, by all authors, as an indication that the organism is overcoming the infection or that it has recovered from it, while a steady fall indicates the reverse.

Rumpf [3] found in his fourteen cases, mostly of phthisis, that the blood alkalinity was not altered materially as long as the general condition of the patients remained good. With the advent of cachexia the alkalinity decreased.

The relation of its increase to the course of the infection is shown also by Strauss.[4] Recovery occurred in two cases of pneumonia, one of typhoid fever and one of erysipelas, in which the alkalinity was markedly above the normal. Five cases of typhoid fever and one case of pneumonia showed no increase, and death occurred in every instance.

A depression of the alkalinity occurs also when bacterial toxins are introduced, and the fall continues when the dose is large enough to prove fatal; a rise follows the depression if the dose is smaller and is recovered from. Cantani [5] and v. Rigler [6] found that an adequate dose of a specific antitoxin, for instance in diphtheria toxin-poisoning, arrests the downward course and promptly increases the blood alkalinity, and the animals recover.

Fodor & Rigler [7] and Emmerich & Löw [8] have asserted a close relation between blood alkalinity and immunity. The former have shown in their investigations that the observed increase is not due to an increase of alkaline salts in the blood, and they hold that, when specific toxins are injected, the subsequent increase of the alkalinity depends upon a vital reaction on the part of the leucocytes which are stimulated specifically to the formation of an alkaline organic substance. Emmerich & Löw support this view and, in their opinion, an increase of the alka-

[3] W. H. Rumpf; Centrlbl. f. klin. Med. 1891, XII, 441.
[4] H. Strauss; Zeitschr. f. klin. Med. 1896, XXX, 317.
[5] A. Cantani; Centrlbl. f. Bakt. 1896, XX, 566.
[6] G. v. Rigler; Centrlbl. f. Bakt. 1901, XXX, 913 and 948.
[7] Fodor & v. Rigler; Centrlbl. f. Bakt. 1897, XXI, 186.
[8] Emmerich & Löw; Zeitschr. f. Hyg., etc. 1901, XXXVI, 9.

linity in immunity is due to this new-formed organic alkaline substance or combination. Karfunkel[9] found, when he administered tubercle bacillus emulsion in slowly increasing doses, that an increase of the blood alkalinity could be observed two hours later, the rise continuing for six hours; but after twenty-four hours the increase had subsided again. In the end he could not show that an increase had occurred over the per cent which was present when the treatment was begun. The results of our determinations from three to eight months after vaccination show, however, that the gain in the blood alkalinity has been maintained in these cases.

* * * * *

The results of bactericidal experiments, made and completed since our report of 1912, with immune sera taken after prophylactic vaccination, or during and after treatment with either the Watery Extract of Tubercle Bacilli or with the Vaccine. appear in the following table. All the immune sera that were used complied, in their titer of complement-fixation and of bacteriolytic action, with the standard which we had previously found to be an efficient guide.[10]

The headings of the individual columns in the tables are self-explanatory. When no time since vaccination is given, the serum was obtained within one month; in the case of treated patients, it was obtained during the time while they were being treated, or within one month after their discharge. An asterisk

[9] KARFUNKEL; Zeitschr. f. Hyg., etc. 1899, XXXII, 149.

[10] Complement-fixation must be complete with 0.05 cc. of inactive serum, diluted with normal salt solution to 1:4, with all four, and diluted to 1:8 with at least two of our antigens, namely, the whole antigen in the form of an emulsion of tubercle bacilli, one protein, and two lipoids; and marked morphological changes must be demonstrable upon virulent tubercle bacilli, after incubating 0.01 mgr. (moist weight) in emulsion free from all clumps, for 24 hours at 37° C., with 0.2 c.c. of the undiluted serum in its fresh, active state. The degree of morphological changes is determined on comparative microscopical examination, by using normal serum or normal salt solution for the incubation of control tests. We express the degrees by (trace); 1 (moderate); 2 (marked); 3 (complete), the latter representing complete disintegration and therefore an entire absence of stained rods.

For the result of experiments with immune sera which did not comply with this standard, or that were not examined completely, the reader is referred to p. 398 ff.

in this column indicates that the patient was treated with the Watery Extract of Tubercle Bacilli.

When the infection was intraperitoneal, the infection site was examined at the point where the needle-puncture had been made, and also the corresponding portion of the parietal peritoneum; the mesenteric and inguinal lymph glands were then considered as regional. After subcutaneous infection, the lymph nodes nearest to, and corresponding with the path of absorption, and after intratracheal infection, the tracheal, cervical and thoracic groups of lymph nodes were considered as regional.

In a tabulation like the present one the autopsy results can of necessity be given only briefly; but we have endeavored to omit nothing of interest that would enable the student to form an opinion of the lesions found. Our own interpretation of the experimental results is tabulated under "General Results."

The findings in internal organs were often obscured by pseudo lesions. When we record such lesions, we give the organs in which they were found; such a record was made usually only in instances in which it was justified by both macroscopical and microscopical examinations of smears and sections.

Under the heading of "smears," we record our findings of tubercle bacilli in caseous lesions and in antiformin residues from other lesions which were at all doubtful on inspection. The same is the case for tubercles found in "sections" some of which were serial, while others were made in duplicate, one of them being stained for tubercle bacilli, the other for tubercles.

No effort was spared in all microscopical work to determine the actual condition, whenever this seemed to require verification; at the same time we avoided superfluous labor when the evidence was otherwise satisfactory and conclusive.

In our interpretation of the results of a given experiment it will be found in certain cases that we record "No tuberculosis," although tubercle bacilli were found in smears, and especially in smears from antiformin residues of fibrous or fibrocaseous lesions, for instance in the guinea-pig under group-number 15. This animal was killed 346 days after infection and was then in excellent condition, weighing 740 grams. Adhesions between the omentum and abdominal wall, and six fibrocaseous nodules were found in the omentum, tubercle bacilli being demonstrated in the adhesions. The nodules in the omentum were hard and

predominantly fibrous with slight central caseation, and we looked upon them as processes of encapsulation and of retrogressive character. Although we made no sections in this particular case, we have often made them in like cases, as a rule without finding histological tubercles. In this animal the presence of these lesions had in no wise interfered with the normal functions and health of the animal, inasmuch as it was well nourished and fat when it was killed, and we considered that these lesions were simply the still visible remains of an infection that had been resisted and overcome successfully, and that the finding of tubercle bacilli in the organized adhesions did not constitute tuberculosis.

That the presence, at the infection site, of caseation with or without tubercle bacilli in smears does not necessarily indicate a lack of resistance, will be admitted by experienced observers. It is confirmed by the findings of Prudden and Hodenpyl [11] in their experiments with dead tubercle bacilli. Like observations have been made by others, and also by ourselves in our studies of the pathogenic action of dead tubercle bacilli, including the finding of caseous and necrotic foci in internal organs. In our experience such lesions developed most frequently, after the injection of dead tubercle bacilli, when the injected dose was large and the bulk of the fluid holding them in suspension was small. The fact that, in the present experiments, the infection had not extended to, and had not produced typical tuberculosis in distant lymph glands and in the viscera may, therefore, be accepted as a proof of the partial, if not complete, destruction of the virulence.

However we may judge of this and of similar findings, we could not consistently class them as indicative of manifest tuberculous disease, inasmuch as the experiments were made for the purpose of showing the bactericidal action of the tested immune sera; this action is amply proved in the particular experiment, regardless of opinions concerning the importance and significance of the changes found on autopsy at the infection site.

In our consideration of pseudo-tuberculosis (p. 341) we have referred to the influence which its presence appeared to have exerted on the occurrence of marasmus or death in our bac-

[11] PRUDDEN AND HODENPYL; New York Med. Journ. 1891, LIII, 697.

tericidal and other studies. In our tabulation we have entered "no apparent cause" for deaths of animals when the lesions found were so slight that they could not be presumed to have stood in direct relation to death.

The deaths which we recorded as due to toxemia were observed almost exclusively after intraperitoneal infection with tubercle bacilli incubated with active immune sera when marked bacteriolytic action had occurred, whereas after subcutaneous infections, as a rule, only a temporary loss of weight of 30 to 50 grams followed. We attribute the difference in the effect between intraperitoneal and subcutaneous infections to the relative rapidity of absorption.

Toxemia occurred also after the subcutaneous injection of a mixture of tubercle bacilli and immune serum, although usually it was recovered from. The animals lost weight during the first few days after infection, but only slight or no losses were noted in the control animals.

The question of a possible causal relation to our results, of bacterial contaminations in our tubercle bacillus emulsions used for infection, was referred to in the discussion of diagnostic inoculations of guinea-pigs, on p. 341; this question may be raised again in this connection, especially as the emulsion of tubercle bacilli was subjected to incubation for twenty-four hours with a nutrient medium like blood-serum which may not be sterile itself, and in which contaminating bacteria can grow and multiply rapidly. Although we admit freely that absolutely sterile work was not possible in any of these experiments, we guarded against contamination with much care, and we do not believe that pathogenic bacteria could have found access to our tubes during incubation or before infection. The microörganisms likely to be introduced by air-infection are saprophytes which do not cause inflammation, abscess or toxemia.

The fact that no such signs of toxemia were noted in our control experiments with human or animal sera, and that most of the principals concerned died in the course of a day or two or even a few hours after infection, speaks against the possibility of contamination. The autopsy findings were not those that would be referable to the action of pathogenic bacteria; they were limited to the lungs which were found anemic, and to the heart which was dilated and [illegible] with blood. In intraperi-

toneal infections, peritonitis was not found in animals which lived several days or longer, and in several instances of early death after this mode of infection the small amount of peritoneal exudate which was found proved to be sterile.

For all these reasons it is highly improbable that our anomalous results were due, or can be attributed with justice, to a bacterial contamination of our tubercle bacillus emulsions or of the sera which were used.

On further examination of our tables, it will be noted that not all of our autopsies were verified by microscopical examinations of smears and sections. These were omitted more frequently before we had become aware of the great prevalence of pseudo-tuberculosis among our animals. That we have not been negligent in this respect, however, becomes evident on comparing published records of other experimenters who frequently appear to have depended upon macroscopical findings alone; this may be sufficient when pseudo-tuberculosis is not a factor to be reckoned with, as will appear in the first and in the last series of bactericidal experiments (see Group-Numbers 1-21 and 129-136). The former experiments were made before pseudo-tuberculosis made its appearance in our animals, and the latter in the spring of 1914, when we happened to be fortunate in obtaining a lot of normal animals; both groups show the same uniformity in results and freedom from complications as we had been able to report them in 1912.

The experiments with immune sera were all controlled properly. The controls exceeded the principals in number, because of our purpose of including the study of sera taken before vaccination or treatment, which made it necessary to undertake experiments with sera from normal animals, and also tests in which the serum was replaced by normal salt solution for the control of virulence of the tubercle bacillus emulsion in the doses which were employed for infection. To make a comparison possible we tabulate the control experiments separately, except those for the bovine infections of rabbits.

I.—A. HUMAN SERA AFTER VACCINATION.—

Group No.	Lab. No.	Mode of Infection	Serum from	Time since Vaccination	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs—Tuberculosis or Lesions suspected of Tuberculosis
					Gm.	*Gm.*		
1	39	i. p.	infant	—	630	680	0	Five hard nodules in liver
2	40	"	child	8 yrs. *)	520	600	0	Three hard nodules in liver
3	41	"	"	—	570	1070	0	Normal
4	42	"	"	—	450	710	0	"
5	45	"	"	8 yrs. *)	670	930	0	"
6	48	"	"	6 mo.	430	430	0	"
7	49	"	"	6 "	600	600	0	"
8	50	"	"	6 "	510	500	0	"
9	51	"	"	6 "	700	700	0	"
10	52a	"	"	6 "	740	560	0	"
11	53	"	"	6 "	450	600	0	"
12	54	"	"	6 "	680	600	0	"
13	56	"	"	6 "	590	320	0	"
14	64	"	"	6 "	720	380	0	Omentum thickened; spleen slightly enlarged
15	65	"	"	6 "	705	740	0	Adhesions; 6 fibrocaseous nodules in omentum
16	67	"	adult	6 "	540	410	0	1 hard nodule in liver
17	68	"	"	—	440	860	0	Normal
18	145	"	child	6 mo.	400	615	0	"
19	175	"	infant	—	230	600	0	"
20	252	"	child	1 mo.	450	500	0	Few hard nodules in omentum
21	253	"	"	1 "	450	560	0	3 hard nodules in omentum
22	254	"	"	1 "	410	400	caseous	Liver enlarged, spleen enlarged, doubtful
23	257	"	"	1 "	410	360	"	Liver and spleen much enlarged
24	273	"	adult	—	380	320	"	Typical hobnail liver. Spleen pseudo
25	276	"	"	—	380	520	0	Normal
26	277a	"	child	1 mo.	390	580	0	Omentum 3 small fibrous nodules, central caseation
27	277b	"	"	1 "	340	680	0	Enlarged bronchial gland, fibrous; central caseation
28	278	"	"	1 "	375	500	0	1 hard nodule in omentum
29	279	"	adult	—	360	280	0	Pseudo of liver, spleen, lungs
30	281	"	"	—	420	400	0	3 nodules, 2 mm. in omentum, fibrous
31	351	"	"	—	360	200	0	Normal
32	384	"	"	—	330	300	0	"
33	408	i. card.	animal	6 mo.	300	300	0	"
34	409	s. c.	child	12 "	380	440	0	4 fibrous nodules in omentum
35	410	"	"	12 "	430	880	0	Spleen slightly enlarged
36	411	"	"	12 "	400	500	caseous	1 yellow nodule in liver
37	412	"	"	12 "	470	410	0	Normal
38	413	"	"	12 "	560	700	0	"
39	414	"	"	12 "	460	770	0	"
40	415	"	"	12 "	420	760	0	Liver, area of necrosis; spleen enlarged. 1 nodule in each, liver and spleen
41	416	"	"	12 "	420	500	0	Liver, caseating areas; lung, cavity
42	417	"	"	12 "	460	560	caseous	3 fibrous nodules in omentum; several in spleen
43	418	"	"	12 "	400	560	0	Normal
44	419	"	"	12 "	380	480	0	Few fibrous nodules in omentum
45	420	"	"	12 "	420	550	0	Normal
46	421	"	"	10 wks.	380	470	0	2 fibrous nodules in each, liver and spleen
47	423	"	"	10 "	430	580	0	Several fibrous nodules in each, liver and spleen
48	425	"	"	10 "	430	680	0	Spleen slightly enlarged
49	426	"	"	10 "	460	690	0	Spleen slightly enlarged
50	427	"	"	10 "	430	630	0	Numerous fibrous nodules in liver and spleen
51	430	"	"	10 "	450	450	0	Spleen slightly enlarged
52	454	"	"	10 "	520	550	0	Diffuse fibrosis in liver and spleen

*Vaccinated with watery extract of tubercle bacilli.

AMBOCEPTORS AND LYSIS REACH STANDARD

[1]) 0 = negative
— = not made

Pseudo Lesions	Cause of Death	Lived, Days	T. B. in Smears [1])	Tubercles, Sections [1])	General Results	Remarks
0	pneumonia	503	0	0	no tbc.	
0	no cause	287	0	—	" "	
0	killed	675	—	—	" "	
0	pastur. seps.	207	—	0	" "	
0	" "	196	—	—	" "	
0	toxemia	12 hrs.	0	0	" "	
0	"	12 "	0	0	" "	
0	"	12 "	0	0	" "	
0	"	20 "	—	—	" "	
0	pastur. seps.	230	—	—	" "	
0	pneumonia	230	0	—	" "	
0	"	230	0	0	" "	
0	no cause	6	0	0	" "	
0	" "	426	0	0	" "	
0	killed	346	posit.	—	" "	
0	pastur. seps.	86	0	—	" "	
0	killed	613	0	0	" "	
0	"	108	—	—	" "	
0	"	169	0	—	" "	
0	strangul. intest.	143	0	0	" "	
0	pneumonia	146	0	0	" "	
liver, spleen	no cause	165	0	0	" "	
" "	pseudo-tbc.	153	0	0	" "	
" "	killed	305	0	0	" "	Autopsy Contr. #1 (G.P. No. 727)
0	"	173	0	0	" "	
0	"	173	posit.	0	" "	
0	"	173	"	0	" "	
0	pneumonia	149	0	—	" "	
liver, spleen, lungs	pseudo-tbc.	84	0	0	" "	
0	pneumonia	93	0	—	" "	
0	no cause	11	0	—	" "	
0	toxemia	few hrs.	0	—	" "	Culture periton. exudate negat.
0	"	"	—	—	" "	
0	sepsis	87	posit.	0	" "	
0	killed	229	0	0	" "	
lung	pneumonia	148	posit.	—	" "	Autopsy Contr. #2 (G.P. #535)
spleen	pseudo-tbc.	207	"	0	" "	
"	killed	167	0	0	" "	Autopsy Contr. #3 (G.P. #574)
"	"	167	0	—	" "	Autopsy Contr. #4 (G.P. #573)
liver	"	229	posit.	posit.	slight tbc.	Autopsy Contr. #5 (G.P. #640)
doubtful	tbc.	235	"	0	chron. tbc.	
"	killed	137	spleen pos.	—	no tbc.	
liver, spleen	"	250	0	—	" "	
liver	trauma	83	posit.	0	" "	
0	intestinal obstruct.	52	0	—	" "	
0	killed	123	0	—	" "	
liver	"	123	spleen pos.	—	" "	
0	"	122	0	—	" "	
0	"	123	0	—	" "	
liver, spleen	"	249	0	0	" "	Autopsy Contr. #6 (G.P. #711)
0	intest. obstr.	66	0	—	" "	
liver, spleen	killed	232	posit.	0	" "	Autopsy Contr. #7 (G.P. #712)

I.—A. HUMAN SERA AFTER VACCINATION.—

Group No.	Lab. No.	Mode of Infection	Serum from	Time since Vaccination	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs— Tuberculosis or Lesions suspected of Tuberculosis
					Gm.	*Gm.*		
53	494	s. c.	child	15 mo.	450	400	0	2 fibrous nodules in omentum; spleen enlarged
54	495	"	"	15 "	460	410	caseous	Areas of fibrosis in liver and spleen
55	496	"	"	15 "	400	700	0	Several doubtful nodules in liver and spleen
56	497	"	"	15 "	430	680	caseous	Several doubtful nodules in liver and spleen
57	498	"	"	15 "	430	500	0	Few hard nodules in liver
58	499	"	"	15 "	470	480	0	Fibrous areas in liver and spleen
59	512	"	"	2 "	560	460	0	Normal
60	517	"	"	—	370	300	0	"
61	523	"	"	20 mo.	300	640	0	Spleen slightly enlarged
62	524	"	"	20 "	520	540	0	" " "
63	525	"	"	20 "	330	620	0	Normal
64	526	"	"	20 "	330	660	0	Spleen slightly enlarged
65	527	"	"	20 "	380	250	0	Necrotic area in liver
66	528	"	"	20 "	320	520	0	Spleen slightly enlarged; liver 1 hard nodule, 3 mm.
67	529	"	"	20 "	480	725	0	Normal
68	530	"	"	20 "	300	600	0	"
69	531	"	"	20 "	310	510	0	One whitish nodule in liver
70	532	"	"	20 "	430	670	0	Normal
71	533	"	"	20 "	400	650	0	"
72	534	"	"	20 "	400	320	0	1 caseous nodule, 2 mm., in horn of uterus
73	546	"	adult	20 "	600	510	0	Normal
74	547	"	child	20 "	300	610	0	1 miliary nodule in liver
75	548	"	adult	20 "	300	210	0	Normal
76	549	"	child	20 "	510	680	0	1 fibrous nodule, 1 mm., in liver
77	550	"	"	20 "	260	600	0	Mesent. gld. 3 mm. hard. 1 nodule in liver
78	551	"	"	20 "	280	700	0	Few doubtful nodules in each lung
79	552	"	"	20 "	300	650	scar	Several fibrous nodules, 1 mm., in spleen
80	560	"	"	—	360	580	0	1 suspicious nodule on gall-bladder
81	568	"	"	—	340	640	0	Several doubtful nodules in liver
82	774	i. p.	"	22 mo.	420	560	0	Few small, hard nodules in spleen
83	775	"	"	22 "	480	540	0	1 nodule, 3 mm., in spleen, central caseation
84	776	"	"	22 "	430	670	0	2 doubtful nodules in left testis
85	777	"	"	22 "	450	600	0	Few doubtful nodules in spleen
86	778	"	"	12 "	450	720	0	1 nodule, 2 mm. in omentum, central caseation
87	779	"	"	24 "	410	290	0	Few ½ mm. nodules in spleen and liver. Mesent. gld. 5 mm. soft
88	780	"	"	24 "	410	680	0	Few hard nodules in spleen
89	781	"	"	24 "	420	440	caseous	8 hard nodules, 2 mm., in omentum
90	782	"	"	24 "	350	270	0	Normal
91	783	"	"	24 "	390	280	0	Caseous nodules in omentum
92	784	"	"	24 "	420	680	0	Normal
93	785	"	"	24 "	430	650	0	"
94	786	"	"	24 "	350	565	0	"
95	787	"	"	1 "	440	550	0	Mesent. gld. enlarged. Spleen slightly enlarged
96	788	"	"	24 "	370	680	0	Few doubtful nodules in spleen
97	789	"	"	1 "	420	650	0	Normal
98	790	"	"	1 "	440	670	0	"
99	791	"	"	22 "	400	700	0	"
100	792	"	"	1 "	380	610	0	1 nodule in spleen, fibrous
101	793	"	"	22 "	350	600	0	Mesent. gld. enlarged, soft; adhesions; spleen few hard nodules
102	794	"	"	22 "	330	650	0	Normal
103	795	"	"	1 "	360	290	0	"

AMBOCEPTORS AND LYSIS REACH STANDARD—*Continued.*

[2]) 0 = negative
— = not made

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears [2])	Tubercles, Sections [2])	General Results	Remarks
0	no cause	9	0	—	no tbc.	
liver, spleen	" "	243	0	—	" "	Autopsy Contr. #8 (G.P. #913)
doubtful	killed	181	posit.	0	" "	Autopsy Contr. #9 (G.P. #720)
liver, spleen	"	156	0	—	" "	
liver	abortion	106	0	—	" "	
liver, spleen	killed	181	posit.	0	" "	{ Autopsy Contr. #10 G.P. #717 { Autopsy Contr. #11 G.P. #719
0	no cause	94	0	—	" "	
0	" "	37	—	—	" "	
0	killed	228	0	0	" "	
0	no cause	67	0	—	" "	
0	killed	228	0	0	" "	
0	"	106	spleen pos.	0	" "	Autopsy Contr. #12 (G.P. #724)
liver	no cause	23	0	—	" "	
0	killed	106	spleen pos.	0	" "	Autopsy Contr. #13 (G.P. #723)
0	"	106	0	—	" "	
0	"	106	—	—	" "	
0	"	92	0	0	" "	
0	"	106	0	—	" "	
0	"	228	0	—	" "	
doubtful	no cause	165	posit.	0	" "	Autopsy Contr. #14 (G.P. #882)
0	intest. strangul.	194	0	—	" "	
0	killed	106	0	—	" "	
0	no cause	41	0	—	" "	
0	killed	227	posit.	—	" "	
0	"	95	0	0	" "	
0	"	227	0	0	" "	
0	"	227	0	0	" "	
0	no cause	95	—	0	" "	
0	killed	98	0	0	" "	
0	"	67	0	posit.	" "	
0	"	67	0	0	" "	
0	"	157	0	0	" "	
0	"	94	0	—	" "	
0	"	94	0	—	" "	
liver, spleen	pneumonia	13	mes. gld. posit.	—	L. gld. tbc.	Autopsy Contr. #15 (G.P. #815)
0	killed	94	posit.	0	" "	
0	intest. obstr.	17	inf. site pos.	—	" "	
0	pneumonia	76	0	0	" "	
0	"	10	posit.	—	spont. tbc.	Genuine spontaneous infection
liver	killed	94	0	—	no tbc.	
0	"	94	0	—	" "	
0	"	94	0	—	" "	
liver	"	94	spleen pos.	0	" "	
spleen	"	160	0	0	" "	
0	"	157	0	—	" "	
liver, spleen	"	160	0	0	" "	
spleen	"	157	0	0	" "	
0	"	157	0	0	" "	
0	"	160	spleen pos.	0	" "	
0	"	160	0	—	" "	
lung	chronic pneumonia	70	0	—	" "	

I.—A. HUMAN SERA AFTER VACCINATION.—

Group No.	Lab. No.	Mode of Infection	Serum from	Time since Vaccination	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs— Tuberculosis or Lesions suspected of Tuberculosis
					Gm.	*Gm.*		
104	796	i. p.	child	22 mo.	370	640	0	Normal
105	797	"	"	22 "	380	320	0	"
106	798	"	"	22 "	400	700	0	"
107	799	"	"	22 "	380	630	0	Spleen slightly enlarged
108	887	s. c.	"	—	580	370	0	4 doubtful nodules in liver
109	888	"	"	—	400	280	congested	Mesenteric gland enlarged
110	889	"	"	—	570	650	0	Normal
111	907	"	"	3 mo.	310	530	0	"
112	1011	"	"	—	380	280	0	1 caseous nodule in each lung-apex
113	1012	"	"	—	360	310	enlarged	Adhesions between lung, pericard. and diaphragm
114	1013	"	"	—	320	205	"	adhesions, lung—pneumonia
115	1016	"	"	—	310	240	"	Normal
116	1161	"	adult	30 mo.	550	350	0	Fibrocaseous area in lung, 5 mm.
117	1165	"	child	30 "	440	380	0	Mesent. gld. enlarged
118	1167	"	adult	1 "	500	420	enlarged	Normal
119	1995	"	child	—	335	290	0	Spleen slightly enlarged
120	1999	"	"	—	390	280	0	Adhesions, lungs
121	2013	"	"	—	440	390	0	Spleen and liver full of nodules. Areas of diffused fibrosis in lungs
122	2015	"	"	—	600	730	caseous	Fibrous areas, 2–5 mm., in liver, spleen, lungs
123	2017	"	"	—	430	320	0	Normal
124	2283	"	adult	31 mo.	510	340	0	Caseous area, 10 mm., and severa nodules in liver
125	2285	"	"	6 wks.	470	340	0	Normal
126	2287	i. p.	child	9 mo.	530	350	0	1 caseous nodule, 1 mm., in omentum
127	2288	"	"		590	380	enlarged	4 nodules, ½ mm., in liver
128	2334	s. c.	"		660	460	0	Normal
129	2339	i. p.	"		650	380	0	Left testicle necrotic
130	2357	"	adult	1 mo.	640	380	0	Normal
131	2358	s. c.	child		300	480	0	"
132	2359	"	"		320	500	0	"
133	2360	"	adult		240	400	0	"
134	2361	"	"		370	510	0	"
135	2378	"	child		320	490	0	"
136	2413	"	"		350	520	0	"
137	2415	"	"		530	690	0	"
138	2416	"	"		410	520	0	"

AMBOCEPTORS AND LYSIS REACH STANDARD—*Continued.*

[2]) 0 = negative
— = not made

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears [2])	Tubercles, Sections [2])	General Results	Remarks
0	killed	157	—	—	no tbc.	
liver, spleen	pseudo-tbc.	11	0	—	" "	
lung	killed	149	0	0	" "	
0	"	160	0	posit.	spleen-tbc.	
liver	no cause	17	0	—	no tbc.	
doubtful	" "	36	0	—	" "	
0	killed	115	—	—	" "	
0	"	114	—	—	" "	
0	no cause	21	posit.	posit.	spont. tbc.	Genuine spontaneous infection
0	" "	24	0	"	" "	" " "
0	pneumonia	20	posit.	0	no tbc.	
0	"	24	0	—	" "	
lung	no cause	22	0	—	" "	
"	" "	26	0	—	" "	
"	" "	15	0	—	" "	
"	" "	21	0	0	" "	
"	" "	24	0	0	" "	
liver, spleen, lung	" "	28	0	0	" "	
liver, spleen, lung	" "	28	0	0	" "	
lung	" "	12	0	0	" "	
liver, lung	" "	22	0	0	" "	
lung	pneumonia	22	0	—	" "	
"	"	23	posit.	0	" "	
"	"	24	0	0	" "	
"	"	8	0	—	" "	
"	"	11	0	posit.	spont. tbc.	Genuine spontaneous infection
liver, lung	no cause	10	0	0	no tbc.	
0	killed	52	0	0	" "	
0	"	58	0	0	" "	
0	"	58	0	0	" "	
0	"	58	0	0	" "	
0	"	45	0	0	" "	
0	"	51	0	0	" "	
0	"	51	0	0	" "	
0	"	51	0	0	" "	

I.—B. HUMAN SERA AFTER TREATMENT.—

Group No.	Lab. No.	Mode of Infection	Serum from	Time since Treatment	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs—Tuberculosis or Lesions suspected of Tuberculosis
					Gm.	*Gm.*		
1	38	i. p.	adult	6 mo.	360	600	0	Spleen slightly enlarged
2	46	"	"	4 mo.	600	660	enlarged	Normal
3	47	"	"		440	650	0	2 fibrous nodules, 2 mm., each in omentum and spleen
4	69	"	"		280	700	0	1 doubtful nodule in spleen
5	243	"	"		460	380	0	Few doubtful nodules in spleen and liver
6	260	"	"	6 mo.	400	370	0	Caseating foci in liver and spleen
7	261	"	"		370	475	0	Normal
8	262	"	"		470	300	0	Liver few small caseous foci; spleen numerous foci, ½ mm.
9	561	"	"		330	365	0	Normal
10	562	"	"		330	400	0	Few nodules in liver; spleen slightly enlarged
11	564	"	"		400	570	0	Numerous nodules in liver and spleen
12	565	"	"		350	630	0	Normal
13	566	"	"		370	550	0	"
14	631	"	"		500	520	0	Area of fibrosis in lower lobe of right lung
15	639	"	"	10 yrs. *)	400	810	mesent. gld. enlg.	Normal
16	647	"	"		450	470	0	"
17	760	s. c.	"		420	730	fibrocas.	Several nodules, 2 mm., in spleen
18	884	"	"	12 mo.	310	260	0	Normal
19	1162	"	"	1 "	665	500	0	"
20	1166	"	"		400	400	0	"
21	1174	"	"		330	250	0	"
22	2019	"	"	30 mo. *)	540	430	0	"
23	2284	"	child	6 mo.	450	290	0	Necrotic area, 15 mm., in liver
24	2362	i. p.	adult		320	570	0	Normal
25	2363	"	"		310	460	0	"
26	2364	"	"		340	600	0	"
27	2365	"	"		280	400	0	"
28	2366	"	"		440	560	0	"
29	2367	"	"		460	560	0	"
30	2411	"	"		380	620	0	"
31	2412	"	"		380	520	0	"
32	2432	"	"		410	560	caseous	"

*Treated with watery extract of tubercle bacilli.

AMBOCEPTORS AND LYSIS REACH STANDARD

[1]) 0 = negative
— = not made

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears [1])	Tubercles, Sections [1])	General Results	Remarks
0	killed	139	0	0	no tbc.	
0	no cause	661	0	0	" "	
0	pneumonia	248	0	0	" "	
0	killed	340	0	0	" "	
lung	no cause	92	0	0	" "	
liver, spleen	pseudo-tbc.	154	0	0	" "	
0	pneumonia	157	0	0	" "	
liver, spleen	no cause	92	0	—	" "	
0	" "	99	0	0	" "	
liver	pneumonia	95	0	0	" "	Autopsy Contr. #16 (G.P. #643)
liver, spleen	killed	190	posit.	0	" "	Autopsy Contr. #17 (G.P. #718)
0	"	102	—	—	" "	
0	no cause	52	0	0	" "	
0	" "	87	posit.	0	" "	Autopsy Contr. #18 (G.P. #883)
0	killed	214	0	—	" "	
0	pneumonia	117	0	0	" "	
spleen	killed	154	0	—	" "	
lung	no cause	68	0	—	" "	
"	abortion	26	0	—	" "	
"	killed	60	0	—	" "	
"	no cause	22	0	0	" "	
lung ?	pneumonia	22	0	0	" "	
lung, liver	no cause	21	0	0	" "	
0	killed	248	0	0	" "	
0	"	58	0	0	" "	
0	"	170	0	0	" "	
0	"	52	0	—	" "	
0	"	52	0	—	" "	
0	"	52	0	0	" "	
0	"	58	0	0	" "	
0	"	52	0	0	" "	
liver, spleen	"	48	infect. site posit.	spleen, posit.	early tbc.	

II. IMMUNE SERA FROM CALVES.—

Group No.	Lab. No.	Mode of Infection	Serum from	After Vaccination	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs— Tuberculosis or Lesions suspected of Tuberculosis
					Gm.	*Gm.*		
1	506	s. c.	Calf, 2	18 mo.	550	680	0	Normal
2	507	"	" 1	18 "	500	500	0	"
3	508	i. p.	" 3	12 "	510	590	0	Spleen slightly enlarged
4	509	"	" 4	12 "	410	660	0	Fibrous nodule in liver
								CONTROLS
5	536	s. c.	rabbit	(normal)	530	350	caseous	Generalized tbc.
6	537	i. p.	"	"	560	370	"	Acute miliary tbc.

III. EXPERIMENTS WITH BOVINE TUBERCLE BACILLI UPON

Rabbit No.	Mode of Infection	Serum From	Taken	Weight Infection	Weight Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs
331	i. p.	child	6 mo. after vacc.	2290	2300	0	Normal
332	"	adult	2½ yrs. aft. vacc.	1610	1630	inguin. glds. enlarged	"
333	"	child	6 mo. after vacc.	1660	1650	0	Ulcer on intest. mucosa. Antiformin residue posit.
336	"	"	8 " " "	1360	1740	0	1 nodule on gastro-hepatic omentum
337	"	"	3 " " "	1180	1720	0	Normal
342	"	adult	6 " " "	2120	1750	0	Adhesive peritonitis
344	"	"	4 " " "	2050	1920	0	" "
345	"	child	2 years " "	1840	1320	0	" "
							CONTROLS
330	"	child	before vacc.	2100	1900	caseous	Generalized tbc.
334	"	rabbit	(normal ser.)	1340	900	0	Miliaries in liver, spleen, omentum
335	"	normal salt sol.	—	1530	1480	caseous	Generalized tbc.
338	"	rabbit	(normal ser.)	1460	1840	mesent. gld. enlarged	Miliaries in liver, spleen omentum
341	"	"	" "	1160	1250	0	" " " "
343	"	normal salt sol.	—	1660	1370	caseous	Generalized tbc.
347	"	rabbit	(normal ser.)	2210	2350	"	" "
348	"	normal salt sol.	—	1960	2020	"	" "
349	"	rabbit	(normal ser.)	2180	2020	0	Enlarged spleen

AMBOCEPTORS AND LYSIS REACH STANDARD

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
0	pneumonia	140	0	—	no tbc.	
lung	no cause	123	0	—	" "	
0	killed	70	0	—	" "	
0	"	132	0	0	" "	
0	tbc.	56	posit.	—	tbc.	
0	"	48	"	—	"	

RABBITS WITH HUMAN SERA AFTER VACCINATION

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
0	killed	51	0	0	no tbc.	
0	"	71	0	0	" "	Coccidiosis
lung	"	76	0	posit.	" "	
0	"	71	0	0	" "	
0	"	76	0	0	" "	
0	"	64	0	0	" "	Had young
0	"	76	0	0	" "	
0	"	76	0	0	" "	
0	tbc.	48	—	—	tbc.	
lung	"	24	posit.	posit.	"	
0	killed	71	"	"	"	
0	abortion	39	"	"	"	
0	trauma	39	"	"	"	
0	killed	64	"	"	"	
0	"	71	"	"	"	
0	"	71	"	"	"	
0	no cause	16	0	"	splean tbc.	

CONTROLS. A.—HUMAN

Group No.	Lab. No.	Mode of Infection	Serum from	Complem.-Fixat. T.B.	Prot.	N.F.	F.A.	Bacteriolysis	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs—Tuberculosis
									Gm.	*Gm.*		
1	52b	i. p.	child	0	—	—	—	—	610	340	caseous	Generalized tbc.
2	55	"	"	0	—	—	—	—	585	400	"	" "
3	61	"	adult	0	—	—	—	—	580	305	"	" "
4	167	"	infant	0	—	—	—	0	345	350	"	" "
5	216	"	child	+	+	+	+	+	360	400	0	" "
6	217	"	"	+	+	+	+	—	330	540	caseous	Fibrocaseous tbc. liver and spleen
7	218	"	"	+	+	+	+	+	370	260	0	Generalized tbc.
8	220	"	"	+	—	—	—	0	320	300	caseous	" "
9	221	"	adult	0	—	—	—	0	300	350	enlarged	" "
10	222	"	child	0	—	—	—	0	345	470	caseous	" "
11	223	"	"	4	—	—	—	0	320	500	0	" "
12	224	"	"	+	—	—	—	0	350	700	0	Liver miliary tbc.—spleen few nodules, 1 mm., lungs free
13	226	"	"	0	—	—	—	0	370	280	enlarged	Generalized tbc.
14	227	"	"	0	—	—	—	0	345	500	"	" "
15	228	"	"	+	—	—	—	0	370	380	caseous	" "
16	229	"	"	0	—	—	—	0	430	630	0	" "
17	230	"	"	+	—	—	—	+	410	520	0	Fibrocaseous lesions in liver and spleen; miliaries in lung
18	236	"	"	—	—	—	—	—	300	250	caseous	Generalized tbc.
19	237	"	"	4	—	—	—	+	300	310	0	Fibrocaseous and miliary tbc.
20	238	"	"	0	—	—	—	0	360	300	caseous	Generalized tbc.
21	239a	"	"	0	—	—	—	0	480	350	"	" "
22	240	"	"	—	—	—	—	—	380	310	"	" "
23	241	"	"	4	—	—	—	+	285	400	"	Tbc. of abdom. organs.—lungs free
24	242	"	"	4	—	—	—	+	400	360	"	Generalized tbc.
25	246	"	adult	0	—	—	—	0	480	280	enlarged	Acute miliary tbc.
26	266	"	"	0	—	—	—	—	470	420	"	Generalized tbc.
27	274	"	"	+	—	—	—	+	350	320	caseous	" "
28	279	"	child	+	—	—	—	0	360	280	enlarged	" "
29	282	"	infant	—	—	—	—	0	310	270	caseous	" "
30	299	"	adult	0	—	—	—	0	450	370	"	" "
31	377	"	child	0	—	—	—	0	620	440	"	Generalized tbc.—fibrocaseous lesions predominating
32	381	"	adult	—	—	—	—	—	470	410	"	Generalized tbc.
33	382	"	child	+	—	—	—	0	470	430	ulcer	Fibrocaseous lesions, lung, liver, spleen, omentum
34	459	s. c.	"	4	+	+	+	1	520	500	0	Omentum thickened
35	462	"	adult	+	—	—	—	+	470	480	0	Omentum thickened; spleen slightly enlarged
36	463	"	child	4	+	+	+	+	730	730	0	3 fibrous nodules in omentum; 2 ditto, in liver.
37	472	"	"	+	—	—	—	—	430	400	caseous	Old tuberculous lesions in mesentery, omentum, liver, spleen, lungs
38	553	"	"	—	—	—	—	—	370	340	open ulcer	Generalized tbc.
39	556	"	"	—	—	—	—	—	340	450	caseous	Chronic tbc. of abdom. organs; few miliar. in lungs
40	558	"	"	0	—	—	—	0	300	410	"	Acute miliary tbc.
41	563	"	"	3	3	3	+	+	360	660	0	Normal
42	602a	"	"	—	—	—	—	+	460	320	caseous	Few tubercles in spleen
43	602b	"	"	+	—	—	—	0	420	400	"	Generalized tbc.
44	605	i. p.	"	3	+	+	+	+	580	560	0	Few nodules, 1 mm., in lung

SERA BEFORE VACCINATION

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
0	tbc.	51	—	—	tbc.	
0	"	47	—	—	"	
0	"	104	posit.	—	"	
0	"	73	"	posit.	"	
0	"	105	—	—	"	
0	killed	205	posit.	—	"	
liver	tbc.	160	"	—	"	
0	"	86	"	posit	"	
0	"	84	"	—	"	
0	"	86	"	—	"	
0	"	132	"	—	"	
0	killed	201	"	posit.	slight tbc.	
0	tbc.	61	"	"	tbc.	
0	"	201	"	—	"	
0	"	82	"	—	"	
0	accident	106	"	posit.	"	
0	killed	198	"	"	"	
0	tbc.	127	—	—	"	
0	"	90	posit.	—	"	
0	"	42	"	—	"	Probably previous spontaneous infection
0	"	74	"	—	"	
0	"	86	"	posit.	"	
0	"	162	"	—	"	
0	"	92	"	—	"	
0	"	38	"	—	"	
0	"	94	"	posit.	"	
liver	"	150	"	—	"	
liver, spleen	"	84	"	posit.	"	
spleen	"	85	"		"	
0	"	84	"		"	
0	"	107	"		"	
doubtful	"	114	"		"	
"	"	205	"		"	
0	killed	221	oment. pos.	—	no tbc.	Autopsy Contr. #19 (G.P. #721). Mother treated with Wat. Ext. of T.B. during pregnancy. Child 6 years old in perfect health. Did not react to Pirquet
0	"	89	spleen pos.	posit.	beginning tbc.	
0	"	428	0	0	no tbc.	Mother treated with Wat. Ext. during pregnancy. Child 4 years old, in good health. No reaction to Pirquet
0	superinfection	12	posit.	—	spont. tbc.	
0	tbc.	127	"		tbc.	
doubtful	"	203	"	posit.	"	
"	"	55	"	"	"	
0	killed	116	—	—	no tbc.	
0	no cause	103	posit.	—	slight tbc.	
0	tbc.	153	"	—	tbc.	
lung	pneumonia	153	0	0	no tbc.	

CONTROLS. A.—HUMAN

Group No.	Lab. No.	Mode of Infection	Serum from	Complem.-Fixat.				Bacteriolysis	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs—Tuberculosis
				T.B.	Prot.	N.F.	F.A.					
									Gm.	*Gm.*		
45	607	i. p.	adult	—	—	—	—	—	520	300	0	Few miliaries in spleen and liver
46	632	"	"	0	—	—	—	0	420	370	enlarged	Generalized tbc.
47	633	"	"	0	—	—	—	0	320	340	caseous	" "
48	812	s. c.	"	0	—	—	—	0	370	210	"	" "
49	813	"	"	++	+	+	+	++	390	620	0	1 nodule in liver
50	814	"	"	+	—	—	—	+	270	410	0	Inguinal lymph glands caseous; miliaries in liver, spleen, lungs
51	818	"	child	0	—	—	—	0	480	380	caseous	Generalized tbc.
52	895	"	"	+	—	—	—	+	380	530	enlarged	" "
53	897	"	"	—	—	—	—	0	370	390	caseous	Chronic tbc.
54	1171	"	adult	—	—	—	—	—	370	410	"	Generalized tbc.
55	1794	"	child	—	—	—	—	—	430	530	"	" "
56	1795	"	"	—	—	—	—	0	540	520	"	" "
57	2030	"	"	0	—	—	—	0	340	270	ulcer	2 areas of old tbc. in lung
58	2031	"	adult	+	—	—	—	0	380	360	"	Spleen slightly enlarged
59	2137	"	"	+	—	—	—	+	560	410	"	Necrotic area in liver—miliaries in spleen and lung
60	2191	"	child	0	—	—	—	0	460	420	0	Generalized tbc.
61	2354	i. p.	"	0	—	—	—	0	520	330	caseous	" "

SERA BEFORE VACCINATION—*Continued.*

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
lung	pneumonia	64	posit.	—	slight tbc.	
0	tbc.	53	"	—	tbc.	
0	"	80	"	—	"	
lung	chronic pneumonia	59	"	—	"	
0	killed	150	"	—	no tbc.	
0	tbc.	102	"	—	tbc.	
0	tbc.	91	"	—	"	
spleen	killed	127	"	—	"	
doubtful	tbc.	145	"	—	"	
"	killed	81	"	posit.	"	
"	"	45	"	—	"	
0	"	65	"	—	"	
spleen	superinfection	26	"	posit.	spont. infection	
liver	killed	26	"	"	tbc.	
"	tbc.	61	"	"	"	
0	"	38	"	"	"	
0	"	45	"	"	"	

CONTROLS. B.—SERA FROM TUBERCULOUS

Group No.	Lab. No.	Mode of Infection	Serum from	Complem.-Fixat. T.B.	Prot.	N.F.	F.A.	Bacteriolysis	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs—Tuberculosis
									Gm.	*Gm.*		
1	225	i. p.	.....	+	+	+	+	+	350	480	caseous	Generalised tbc.
2	265a	"	.....	0	0	0	0	0	350	280	enlarged	Miliary tbc.
3	266	"	.....	0	0	0	0	0	470	420	"	" "
4	267	"	.....	+	+	+	+	0	290	430	caseous	Generalized tbc.
5	268	"	.....	+	+	+	+	0	410	350	"	" "
6	275	"	.....	+	+	+	+	0	300	310	"	" "
7	280	"	.....	0	0	0	0	0	370	330	"	" "
8	291	"	.....	0	0	0	0	0	400	340	"	" "
9	292	"	.....	0	0	0	0	0	410	350	"	" "
10	298	"	.....	0	0	0	0	0	430	320	"	" "
11	300	"	.....	0	0	0	0	0	430	380	"	" "
12	356	"	.....	0	0	0	0	0	280	180	tubercles	Acute miliary tbc.
13	358	"	.....	+	+	+	+	+	320	240	caseous	Generalized tbc.
14	376	"	.....	0	0	0	0	0	370	300	"	" "
15	394	s. c.	.....	+	+	+	+	+	450	600	0	Chronic tbc. of abdominal and thoracic organs
16	395	"	.....	+	+	+	+	+	470	430	caseous	Chronic tbc. pulmonary cavity
17	405	"	.....	0	0	0	0	0	440	470	"	Chronic tbc. abdominal and thoracic organs
18	453	"	.....	0	0	0	0	0	410	380	enlarged	Generalized tbc.
19	460	"	.....	0	0	0	0	0	510	600	caseous	" "
20	461a	"	.....	4	+	+	+	+	600	790	scar	Few fibrous nodules in liver and lungs
21	606a	i. p.	.....	3	+	+	+	+	520	470	0	Omentum thickened; adhesions
22	634	"	.....	0	−	−	−	0	350	210	caseous	Generalized tbc.
23	744	s. c.	.....	+	−	−	−	+	370	530	enlarged	Chronic tbc. of spleen and lungs
24	745	"	.....	+	+	+	+	0	420	520	caseous	Generalized tbc.
25	746	"	.....	+	+	+	+	0	390	320	"	Generalized tbc.
26	747	"	.....	4	−	−	−	+	400	550	enlarged	Miliary of spleen and lungs
27	750	"	.....	+	−	−	−	0	400	420	"	Chronic tbc. abdominal organs
28	752	"	.....	+	−	−	−	0	470	560	caseous	Miliaries in liver and spleen
29	1749	"	.....	+	−	−	−	0	620	540	"	Generalized tbc.
30	1750	"	.....	−	−	−	−	−	630	520	"	Miliaries in liver and spleen
31	2395	"	.....	4	+	+	+	++	400	400	"	Chronic tbc. abdominal organs
32	2410	"	.....	+	−	−	−	0	460	580	"	Spleen, miliary tubercle in lung
33	2434	"	.....	0	−	−	−	0	360	300	"	Generalized tbc.

PATIENTS.—TAKEN BEFORE TREATMENT

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
0	killed	100	posit.	posit.	tbc.	
liver	tbc.	58	—	—	"	
"	"	93	—	—	"	
0	"	173	posit.	—	"	
0	"	112	"	posit.	"	
doubtful	"	210	"	—	"	
spleen	"	105	"	posit.	"	
0	"	85	"	—	"	
0	"	137	"	—	"	
0	"	298	"	—	"	
0	"	106	"	—	"	
0	"	31	"	—	"	
liver	"	81	"	posit.	"	
"	"	100	—	—	"	
0	killed	269	posit.	—	"	
0	tbc.	281	"	posit.	"	
liver	"	153	"	"	"	
spleen ?	"	64	"	—	"	
"	"	107	"	—	"	
0	killed	405	0	0	no tbc.	Sera from same patient
0	no cause	25	oment. pos.	—	" "	Autopsy Contr. #20 (G.P. #646)
0	tbc.	46	posit.	—	tbc.	
doubtful	no sufficient cause	131	—	—	slight tbc.	
liver	tbc.	127	posit.	—	tbc.	
doubtful	"	123	"	—	"	
"	"	172	"	—	"	
0	"	164	"	—	"	
0	killed	38	"	—	"	
0	"	54	"	—	"	
0	"	55	"	—	"	
0	tbc.	170	"	posit.	"	
liver	killed	51	"	"	"	
lung	tbc.	121	"	"	"	

C.—CONTROLS WITH

Group No.	Lab. No.	Mode of Infection	Serum from	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glands	Internal Organs—Tuberculosis
1	245	i. p.	G. pig	380	250	enlarged	Generalized tbc.
2	247	"	"	480	300	caseous	" "
3	1796	s. c.	"	370	260	"	" "
4	1797	"	"	440	460	"	" "
5	2001	"	"	340	260	"	Miliary tbc. liver, spleen, lungs
6	2002	"	"	550	470	"	Generalized tbc.
7	2003	"	"	340	240	"	Miliary tbc., liver, spleen, lung
8	2005	"	"	530	380	"	" " "
9	2006	"	"	370	260	"	" " "
10	2020	"	"	400	350	"	" " " spleen
11	2021	"	"	310	280	"	" " " lung
12	2023	"	"	320	250	"	" " " " spleen
13	2024	"	"	330	280	ulcer	" " " " "
14	2025	"	"	400	260	caseous	Generalized tbc.
15	2026	"	"	370	320	"	Miliary tbc., liver, spleen, lung
16	2027	"	"	340	200	"	" " " " "
17	2032	"	"	370	270	"	" " " " "
18	2033	"	"	340	230	"	" " " " "
19	2034	"	"	350	240	"	" " " " "
20	2195	"	"	380	320	"	Generalized tbc.
1	2139	"	Rabbit	720	520	"	" "
2	2245	i. p.	"	650	no record	"	4 necrotic areas in liver
3	2289	"	"	570	530	"	Generalized tbc.
4	2291	"	"	510	420	"	Miliary acute tbc.
5	2295	"	"	620	600	"	Generalized tbc.
6	2297	"	"	530	630	"	" "
7	2299	"	"	500	300	"	Miliary tbc.
8	2345	"	"	650	360	0	2 necrotic foci in liver
9	2346	"	"	560	380	0	7 mm. area in liver; left testicle necrotic
10	2347	"	"	610	390	0	Normal
11	2340	"	"	680	370	0	"
12	2368	"	"	350	480	caseous	Generalized tbc.
13	2379	"	"	320	380	"	" "

ANIMAL SERA

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
0	tbc.	40	posit.	—	tbc.	
0	"	35	"	—	"	
0	superinfection	47	"	posit.	"	Superinfection 0.01 mg. T.B., Died 1 day later.
0	"	90	"	"	"	Superinfection like No. 3. Died on 12th day.
doubtful	tbc.	32	"	—	"	
liver	"	39	"	posit.	"	
"	killed	43	"	"	"	
lung	tbc.	30	"	0	"	
0	"	31	"	posit.	"	
lung	no cause	30	"	"	"	
0	"	28	"	"	"	
0	killed	30	"	"	"	
0	tbc.	47	"	"	"	
lung, liver	"	47	"	"	"	
0	killed	23	"	"	"	
0	tbc.	31	"	—	"	
0	"	44	"	—	"	
0	"	54	"	posit.	"	
doubtful	"	44	"	—	"	
0	"	45	"	posit.	"	
spleen	killed	113	posit.	posit.	tbc.	
liver	pneumonia	11	—	—	no tbc.	
spleen	killed	75	posit.	posit.	tbc.	
0	tbc.	38	"	"	"	
0	killed	71	"	"	"	
liver	"	75	"	—	"	
0	tbc.	42	"	posit.	"	
liver	pneumonia	11	—	—	no tbc.	
"	"	8	—	—	"	
"	"	10	—	—	"	
0	"	11	—	—	"	
liver	killed	52	posit.	posit.	tbc.	
0	"	45	"	"	"	

CONTROLS. D.—TUBERCLE BACILLI

Group No.	Lab. No.	Mode of Infection	Weight at Infection	Weight at Autopsy	Infection Site Reg. Lymph Glds.	Internal Organs
1	65b	i. p.	680	360	enlarged	Acute miliary tbc.
2	67b	"	730	405	"	" " "
3	73b	"	380	310	caseous	Generalized tbc.
4	146	"	420	300	"	" "
5	172a	"	400	280	"	Acute miliary tbc. of abdominal organs
6	178b	"	330	390	"	Generalized tbc.
7	179b	"	300	360	"	" "
8	180b	"	350	410	"	" "
9	279b	"	300	310	"	" "
10	378	"	480	310	"	" "
11	461b	s. c.	395	310	"	" "
12	554	"	370	480	"	" "
13	554	"	330	570	"	" "
14	557	"	450	360	"	" "
15	558	"	300	410	"	" "
16	559	"	430	280	"	" "
17	606b	"	300	260	"	Miliary acute tbc.
18	761	i. p.	360	450	"	" " "
19	762	"	340	270	enlarged	Generalized tbc.
20	763	"	330	340	caseous	Miliary tubercle in liver, spleen, lungs
21	764	"	390	345	enlarged	" " " " " "
22	765	"	370	430	"	" " " " " "
23	767	"	390	370	caseous	Generalized tbc.
24	768	"	450	410	enlarged	Few miliaries in spleen
25	769	"	400	580	caseous	Generalized tbc.
26	770	"	360	450	"	" "
27	771	"	400	530	"	" "
28	772	"	400	480	"	" "
29	773	"	380	310	"	" "
30	821	s. c.	500	500	"	" "
31	823	"	310	250	"	" "
32	827	"	400	510	"	" "
33	872	"	350	295	"	Miliary tubercles in abdominal organs
34	873	"	360	420	enlarged	Miliary tubercles abdom. org.; few miliaries in lungs
35	878	"	305	450	"	Miliary tubercles abdom. org.
36	881	"	440	420	caseous	Generalized tbc.
37	885	"	310	510	"	" "
38	906	"	400	400	"	" "
39	908	"	320	275	"	" "
40	910	"	500	580	"	Miliaries in spleen
41	912	"	500	590	"	" " "
42	931	"	450	280	"	Miliaries in spleen; old caseating lesion 10 mm. in lung
43	932	"	360	250	"	Miliaries in liver and lungs
44	933	"	430	340	"	Miliaries in liver and spleen
45	935	"	450	280	"	Acute miliary tbc.
46	936	"	350	300	"	" " "
47	937	"	340	320	"	" " "
48	991	"	425	310	"	" " "
49	1018	"	300	330	"	" " "
50	1020	"	350	300	"	" " "
51	1022	"	290	260	"	Miliaries in abdom. organs—few in lungs
52	1715	"	360	360	"	Miliaries in abdom. organs; tuberculous pneumonia
53	1751	"	490	430	"	Generalized tbc.
54	1752	"	510	420	"	Acute miliary tbc. abdom. org.; tubere. pneumonia
55	1760	"	575	430	"	Generalized tbc.
56	1761	"	375	310	"	" "
57	2290	i. p.	630	350	"	" "
58	2292	"	620	400	"	" "
59	2296	"	580	330	"	" "
60	2298	"	630	460	"	" "

INCUBATED WITH NORMAL SALT SOLUTION

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
0	tbc.	42	—	—	tbc.	
0	"	36	posit.	—	"	
0	"	73	"	—	"	
0	"	58	"	—	"	
liver	"	35	"	—	"	
0	"	60	"	—	"	
0	"	81	"	—	"	
0	"	53	"	—	"	
liver	"	84	"	—	"	
"	"	57	"	—	"	
0	"	74	"	—	"	
0	"	106	"	—	"	
0	killed	94	"	—	"	
0	tbc.	104	"	—	"	Autopsy Contr. #21 (G.P. #2120)
0	"	55	"	—	"	
0	"	125	"	—	"	
0	"	56	"	—	"	
liver	"	24	"	posit.	"	
0	"	76	"	"	"	
0	"	67	"	—	"	
0	"	56	"	—	"	
0	killed	67	"	—	"	
doubtful	tbc.	51	"	—	"	
liver	pneumonia	11	"	—	"	
0	killed	94	"	—	"	
0	"	94	"	—	"	
0	"	94	"	—	"	
lung	"	94	"	—	"	
0	tbc.	64	"	—	"	
0	"	95	"	—	"	
0	"	70	"	—	"	
0	"	105	"	—	"	
lung	"	37	"	—	"	
0	"	43	"	—	"	
liver	"	41	"	—	"	
0	"	92	"	—	"	
0	"	76	"	—	"	
doubtful	"	112	"	—	"	
"	"	35	"	—	"	
0	killed	36	"	—	"	
lung	"	35	"	—	"	
0	superinfection ?	45	"	posit.	"	
lung	tbc.	48	"	—	"	
ung ?	pneumonia	53	"	—	"	
0	tbc.	38	"	—	"	
0	"	43	"	—	"	
0	"	57	"	—	"	
0	"	85	"	—	"	
0	"	94	"	—	"	
0	"	47	"	—	"	
0	"	68	"	—	"	
0	"	57	"	posit.	"	
0	"	57	"	"	"	
0	"	35	"	"	"	
doubtful	"	54	"	"	"	
"	"	41	"	—	"	
spleen	"	46	"	posit.	"	
lung, liver	pneumonia	39	mes. gld.	"	lymph. gld. tbc.	
0	tbc.	71	posit.	"	tbc.	
liver	"	74	"	"	"	

CONTROLS. D.—TUBERCLE BACILLI

Group No.	Lab. No.	Mode of Infection	Weight at Infection	Weight at Autopsy	Infection Site, Reg. Lymph Glds.	Internal Organs
61	2355	i. p.	630	300	caseous	Acute miliary tbc.
62	2369	s. c.	310	420	"	Generalised tbc.
63	2370	"	340	500	"	" "
64	2371	"	340	460	"	" "
65	2372	"	340	430	"	" "
66	2373	"	330	430	enlarged	" "
67	2374	"	440	560	caseous	" "
68	2376	i. p.	320	410	"	" "
69	2377	"	350	380	"	" "
70	2380	"	310	390	"	" "
71	2417	s. c.	410	540	"	" "
72	2418	"	380	490	"	" "
73	2419	"	410	520	"	" "
74	2420	"	390	510	"	" "
75	2421	"	680	580	"	" "
76	2422	"	380	410	"	" "
77	2423	"	360	370	"	" "
78	2424	"	410	290	"	" "
79	2425	"	510	640	"	" "
80	2426	"	400	550	"	" "
81	2427	"	380	430	"	" "
82	2428	"	380	510	"	" "
83	2429	"	360	520	"	" "
84	2430	"	340	390	"	" "
85	2435	"	430	530	"	Miliaries in liver, spleen, lungs
86	2436	"	480	580	"	Miliaries in liver, spleen; few in lungs
87	2517	i. p.	630	740	"	Few tubercles in periton. Omentum thickened. Many nodules, ½–1 mm.
88	2530	s. c.	790	920	"	Normal

INCUBATED WITH NORMAL SALT SOLUTION.—*Continued.*

Pseudo Lesions	Cause of Death	Lived, Days	T.B. in Smears	Tubercles, Sections	General Results	Remarks
0	tbc.	45	posit.	posit.	tbc.	
0	killed	58	"	"	"	
0	"	58	"	"	"	
0	"	58	"	"	"	
0	"	58	"	"	"	
0	accident	32	"	"	"	
0	killed	58	"	"	"	
0	"	52	"	—	"	
0	"	52	"	posit.	"	
0	"	45	"	"	"	
0	"	51	"	"	"	
0	"	51	"	"	"	
0	"	51	"	—	"	
0	"	51	"	posit.	"	
0	"	44	"	"	"	
0	"	51	"	"	"	
0	"	51	"	"	"	
0	tbc.	44	"	"	"	
0	killed	58	"	"	"	
liver	"	51	"	"	"	
0	"	51	"	"	"	
0	"	51	"	"	"	
0	"	51	"	"	"	
0	"	51	"	"	"	
0	"	48	"	"	"	
0	"	48	"	"	"	
0	"	24	"	"	"	
lung	"	24	—	(infect. site posit.)	no tbc.	

AUTOPSY.—CONTROLS FOR

No.	G. P. No.	Infected With Suspected Tissue in Saline Emulsion	From G. P. No.	Mode of Infection	Weight at Infection	Weight at Autopsy	Infection Site Reg. L. Glds.
1	727	Sal. emuls. spleen	273	s. c.	330	700	caseous
2	535	" " liver and spleen	411	"	520	500	0
3	574	" " spleen	413	"	360	300	caseous
4	573	" " "	414	"	350	460	"
5	640	" " "	415	"	450	430	scar
6	711	" " "	427	"	370	500	0
7	712	" " "	454	"	390	330	0
7a	713	" " liver	454	"	370	370	0
8	913	" " "	495	"	290	250	0
9	720	" " "	496	"	330	300	0
10	717	" " "	499	"	380	370	caseous no T.B.
11	719	" " spleen	499	"	480	700	0
12	724	" " "	526	"	370	400	caseous
13	723	" " liver	528	"	360	660	0
14	882	" " uterus	534	"	320	710	0
15	815	" " liver	779	"	350	300	0
16	643	" " "	562	"	530	580	0
17	718	" " spleen	564	"	310	430	0
18	883	" " lung	631	"	370	530	0
19	721	" " omentum	459	"	330	720	0
20	646	" " "	606a	"	450	340	0
21	2120	" " spleen	557	"	270	150	0

BACTERICIDAL EXPERIMENTS

Internal Organs Tuberculous or Suspected Lesions	Pseudo-Tbc.	Cause of Death	Lived, Days	T.B. in Smears	Tubercles in Sections	General Results
Few yellow nodules in liver, many in spleen	liver, spleen	killed	189	inf. site	reg. l. gld.	lymph gld. tbc
Miliary nodules in liver and spleen	"	abortion	57	0	0	no tbc.
White, hard miliary nodules in mesentery, omentum, liver, spleen	abdomin. organs	pseudo-tbc.	79	0	0	" "
Necrotic and caseous foci, liver, spleen lung	liver, spleen lung	"	189	0	0	" "
Liver and spleen doubtful	liver, spleen	killed	239	0	0	" "
Normal	0	"	215	0	0	" "
Numerous nodules, ½ mm., in liver and spleen; necrotic area in left lung	liver, spleen	pseudo-tbc.	109	0	0	" "
Many necrotic foci in lung	lung	"	76	0	0	" "
Old caseous and miliary nodules in lung	0	spont. tbc.	33	posit.	posit.	spont. tbc.
Normal	0	killed	190	0	0	no tbc.
Many yellow nodules in liver, induration in spleen	liver	pseudo-tbc.	105	0	0	" "
Normal	0	killed	190	0	0	" "
Omentum and right testicle	doubtful	pseudo-tbc.	174	0	0	" "
Normal	0	killed	190	0	0	(Note 1) no tbc.
"	0	"	128	0	0	" "
"	0	no cause	132	0	0	" "
"	lung	killed	239	0	0	(Note 2) no tbc.
"	liver, lung	"	190	0	0	" "
"	0	"	128	0	0	" "
"	0	"	190	0	0	" "
"	lung, spleen	pneumonia	53	0	0	" "
"	lung	pseudo-tbc.	11	0	—	" "

Note 1—Lost 330 gm. in the course of 4 days, without apparent cause.
Note 2—Lost 300 gm. in the course of 12 days, without apparent cause.

SUMMARY OF RESULTS

Our tabulation includes 182 experimental infections in which tubercle bacilli were incubated with immune sera. The tubercle bacilli were of human origin in 174 and of bovine origin in 8 of the experiments. The sera used were obtained at various periods after vaccination in 146, and after treatment in 32 cases; sera from immunized animals were used in 4 of the experiments. In order to prevent erroneous conclusions, we exclude from consideration those experiments in which the animals died from other causes or without an apparent cause, before the 30th day after their infection; none of them showed evidence of successful infection. With these omissions, the total results shown by the autopsies are as follows:

In the 182 bactericidal experiments with immune sera, 37 animals died before the 30th day after infection, leaving 145 experiments to be accounted for.

50 animals died from 36 to 510 days after infection, average 165 days.

95 animals were killed, 45 to 675 days after infection, average 147 days.

No tuberculosis was found in.....141, or 97.3 per cent.
Tuberculosis was found in..... 4, or 2.7 per cent.

Our tabulations include 226 control infections. The tubercle bacilli were incubated with human sera taken before vaccination or treatment in 95; with normal animal sera in 40, and with normal salt solution in 91 of the experiments. We make the same deduction on account of deaths which occurred prior to the 30th day, as we did for the principals. In several of the animals which are excluded for this reason, autopsy showed caseous infection sites and enlarged or caseous lymph glands, and in a few of them the infection had extended to internal organs. After this deduction there are 209 control experiments to be accounted for, the autopsies of which gave the following results:

a. 62 human sera taken before vaccination. 3 animals died before the 30th day after infection, leaving 59 experiments to be considered.

47 animals died 38 to 205 days after infection, average 97 days.
Tuberculosis was found in.....46, or 97.8 per cent.
Tuberculosis was not found in..... 1, or 2.2 per cent.
12 animals were killed, 45 to 428 days after infection, average 160 days.
Tuberculosis was found in..... 8, or 66.6 per cent.
Tuberculosis was not found in..... 4, or 33.3 per cent.

b. 33 human sera taken before treatment. 1 animal died before the 30th day, leaving 32 experiments to be considered.

25 animals died 31 to 298 days after infection, average 121 days.
Tuberculosis was found in.....25, or 100 per cent.
7 animals were killed 38 to 405 days after infection, average 139 days.
Tuberculosis was found in..... 6, or 85.7 per cent.
Tuberculosis was not found in..... 1, or 14.3 per cent.

c. 40 sera from normal animals. 9 animals died before the 30th day, leaving 31 experiments to be considered.

22 animals died 30 to 90 days after infection; average 43 days.
9 animals were killed 30 to 113 days after infection, average 66 days.
Tuberculosis was found in.....31, or 100 per cent.

d. 91 normal salt controls. 4 animals died before the 30th day, leaving 87 experiments to be considered.

53 animals died 32 to 125 days after infection, average 61.5 days.
34 animals were killed 35 to 94 days after infection, average 60 days.
Tuberculosis was found in.....87, or 100 per cent.

Tuberculosis was found on autopsy in four principals, Nos. 40, 41, 107 in Table I, A, and No. 32 in Table I, B. The genuine lesions in Guinea-pig No. 40 were slight, being confined to one nodule each in liver and spleen, in which tubercle bacilli were found and sections from which were shown to contain ical tubercles. The infection site was not found and the

regional lymph glands were not enlarged. The necrotic area in the liver was of pseudo origin. The condition of the animal was good, as it weighed 760 grams when it was killed, 229 days after infection. Its autopsy control (No. 5, p. 374), infected with a saline emulsion from the spleen, was free from tuberculosis on being killed 239 days later.

Guinea-pig No. 41 was killed 235 days after infection; on autopsy the infection site was not found, the regional lymph glands were not enlarged. Massive caseation was present in liver and lung, and the latter was the seat of a cavity. Tubercle bacilli were found in smears from the lesions of the liver and in the contents of the pulmonary cavity; but sections from liver and lung, including the walls of the cavity, showed no typical structure of tubercle.

Guinea-pig No. 107 showed no macroscopic tuberculosis, but typical tubercles were found in sections of the slightly enlarged spleen. The infection site was not discovered and the regional lymph glands were not enlarged. The animal had gained 250 grams since its infection, when it was killed 160 days thereafter.

Guinea-pig No. 32 had pseudo lesions in liver and spleen; the infection site was caseous, and tubercle bacilli were found in smears. None were seen in the antiformin residue of the pseudo lesions of liver and spleen; but typical tubercles were present in sections from the latter organ. The control animal, killed at the same time (infected with tubercle bacilli incubated with normal salt solution, G. P. No. 85), had typical miliary tuberculosis of liver, spleen and lungs.

We are inclined to believe that, with the exception of Guinea-pig No. 32, our infections had no relation to the tuberculous lesions found at autopsy.

On referring to the tables, it will be noted that in the four experiments with the sera taken from two immunized calves, and in the eight experiments with tubercle bacilli of bovine origin upon rabbits, the animals were found free from tuberculous disease at autopsy.

In the control experiments with human sera we find that there were six animals which resisted our infections completely. The sera used in these experiments showed
cific amboceptors, and a small degree of

served. In the experiments with sera taken before vaccination we find that, in Guinea-pigs Nos. 34 and 36, the sera were obtained from children, six and four years old, whose mothers had been treated, during gestation, with Watery Extract of Tubercle Bacilli, on account of pulmonary tuberculosis. Both children were in good health and free from tuberculosis as far as our examinations could determine; they failed to react to a cutaneous tuberculin test, and were not vaccinated.

The serum used in Experiment No. 41 was obtained from a girl, fourteen years old, who had been exposed to infection. She had slight changes in both apices, reacted to tuberculin, and showed a marked local and focal reaction to a dose of 3 mgr. of vaccine. The animal experiment, with her serum obtained after vaccination, is recorded in Table IA, No. 81. This girl had gained 19 pounds in six weeks.

The serum used in Experiment No. 44 was obtained from a girl, eight years old, whose mother had died of tuberculosis soon after the child's birth. She appeared to be in good health and no evidence of tuberculosis was found on examination; she reacted, however, to the v. Pirquet test and also showed a marked local reaction to 2 mgr. of vaccine, but no general reaction occurred. The experiment with serum taken from the same child, three months after vaccination, is recorded in Table IA under No. 111, and under No. 38 of the normal salt controls.

The serum used in Experiment No. 49 was obtained from a woman thirty-five years of age, who was in good health and nutrition; her sister was a patient under treatment for pulmonary tuberculosis. Examination of the lungs was negative; she reacted locally and generally to 6 mgr. of vaccine. The normal salt control of this experiment (No. 30) died of generalized tuberculosis.

Of particular interest appears to us the result of the Experiment No. 20 with serum taken before treatment (controls B), in the autopsy of which no tuberculosis was found when the animal was killed 405 days after infection, whereas in the Experiment No. 19, in which the tubercle bacilli were incubated with serum from the same patient, taken on the previous day, the animal died of generalized tuberculosis 107 days after infection; the normal salt control for both (Experiment No. 11) likewise died of generalized tuberculosis, 74 days after infection. In No. 19 the se-

rum showed no specific bacteriolytic amboceptors, while they were present in No. 20, although not in maximal amounts. We can account for the difference in the results only by the absence of specific bacteriolytic amboceptors in the one serum, which corresponds apparently with a negative phase, and by their presence in the other. These two experiments with sera obtained from the same patient, one day apart, and conducted alike in every way, present an interesting illustration of the cyclical appearance and disappearance, in the circulation, of bactericidal and bacteriolytic substances.

Judging of the bactericidal action of human sera taken before vaccination or treatment, from the duration of life in the animals which died spontaneously, we find in the case of human sera that death occurred in 72 of the animals after an average duration of life of 108 days; whereas in 75 animals in which normal sera from guinea-pigs or rabbits, or normal salt solution, had been employed for incubation, the average duration of life was only 57 days; no difference was manifest in favor of the sera of normal animals over the salt solution in the control animals.

The tabulation of the several autopsy controls is self-explanatory. The object of making these experiments was to verify our findings, especially in regard to the genuine or pseudo nature of some of the lesions, or to the virulence of tubercle bacilli, when these were found in apparently healed or healing lesions. It will be noted that the only positive result which we obtained was from a slightly enlarged spleen of Experiment No. 24, recorded in Table IA (p. 352).

On comparing the results of these experiments, in the principals and in all controls, we find that, when tubercle bacilli were incubated with immune sera, tuberculosis was found in 2 per cent, no tuberculosis in 98 per cent. When tubercle bacilli were incubated with human sera taken before vaccination or treatment, tuberculosis was found in 95 per cent, no tuberculosis in 5 per cent; when tubercle bacilli were incubated with normal serum from animals or with normal salt solution, tuberculosis was found in 100 per cent of the autopsies.

We also undertook experiments in animals, intended for a further study of the relation of positive complement-fixation tests to the bactericidal action of the respective immune sera.

These experiments were designed to ascertain whether it was really essential for their uniform success that a maximal amboceptor or lytic action be present in a particular serum-specimen; whether we could be governed in this respect by the results obtained with less than four antigens, or by the degree of morphological changes as demonstrable *in vitro*, without complement-fixation tests being made at all, or in combination with a complement-fixation test made with only one antigen. All these inquiries were desirable because of the large amount of time and labor necessary to make the complete tests, and because we wished to reduce this time, if it were possible without jeopardizing the uniformity and reliability of the results.

Our study with reference to the antibody content of the sera used showed that the degree of morphological alterations in the tubercle bacilli, after they had been subjected to the action of fresh immune serum, and the titer of the immune serum present a close relation and that, as a rule, they correspond.

We noted only four failures when the complement-fixation titer was as high as 1:8 with one antigen, with the degree of lysis being below "2." On the other hand, the bactericidal action was sufficient to prevent the development of tuberculosis with a complement-fixation titer of 1:4, when the lytic action was well marked, as expressed by "2," and we found the bactericidal action equally sufficient when the complement-fixation test had been omitted entirely and the lytic action had been equally well marked or had exceeded the degree expressed by "2."

With the sera showing lower degrees of bacteriolytic action than "1 plus," or complement-fixation less than 1:4, we had almost uniform failures. In 15 trials with a degree of complement-fixation less than 1:4, but associated with a bacteriolytic action of "1 plus," we had 10 successes, 1 complete failure and 4 doubtful results.

With sera showing a higher degree of bacteriolytic action, that is to say, with one of "2" or "3," we experienced no failures in our experiments.

Our general results were as follows:

Tuberculosis due to our infection in	**41**	**39.4**	**per cent**
Tuberculosis doubtful as to our infection	**8**	**7.7**	**" "**
Tuberculosis spontaneous, prior to our infection	**7**	**6.7**	**" "**
No tuberculosis	**48**	**44.8**	**" "**

In these experiments, pseudo-tuberculosis was found in 57 animals, or in 55 per cent of the total number. From these results it appears that the degree of morphological changes caused by the active serum stands in relation to that of its bactericidal action, and that this might be used as a guide by itself, since no failure has occurred in the related experiments when a marked morphological change could be shown in the tubercle bacilli. We appreciate fully, however, that our classification in the degrees of morphological changes occurring under the influence of immune serum is arbitrary and relative, and that individual observers might easily differ in their respective readings.[12]

ACTIVE IMMUNIZATION OF ANIMALS

While we fully appreciate that the successful resistance of a guinea-pig, rabbit or other lower animal to tuberculosis-infection, after treatment with a view to its immunization against the infection, cannot in itself supply incontestable evidence that human subjects would respond in the same manner, we hold that positive results in the animal experiment have a confirmatory value, that they serve to support clinical observations upon human subjects after their vaccination or treatment with the same remedy, and that they corroborate the results of bactericidal experiments made with human immune sera.

If biological tests with human and animal sera give the same evidences of specific antibodies, i.e., if as a rule they are negative before, and positive after the administration of the remedy, or if the bactericidal power of these sera shows a like or a similar relation, we are justified in assuming a like or a similar response to the remedy employed. Failures, particularly in small

[12] In the light of the difficulties which we have encountered in obtaining normal animals, we are considering the feasibility of substituting for our present methods of demonstrating the bactericidal action of immune sera upon animals, the method followed for this purpose with other pathogenic bacteria, namely, the study of the growth and multiplication of tubercle bacilli upon artificial media after they have been subjected to the action of immune serum; and we likewise intend to try Abderhalden's optical and dialysation tests with a view of studying the bacteriolytic action of immune sera upon tubercle bacilli, which, if successful, would enable us to remove the personal equation which adheres to our present method.

laboratory animals like guinea-pigs, in no wise controvert successes in the human subject. E. Albrecht [13] expressed the same views in his theses on the problems of human tuberculosis, when he formulated his general thesis No. 2 as follows: "*The results of animal experiments possess the value of analogous investigations for the problems of human tuberculosis. These results are valid only in so far as they correspond with the established results of human pathological anatomy. Where they differ, the result of the animal experiment is irrelevant.*"

The animal experiment is of value, since experiments upon human subjects are impossible,[14] and clinical observations may be misleading until a comparatively large experience is at hand. The more closely the results of the experiments correspond with obser-

[13] EUGEN ALBRECHT; Frankf. Zeitschr. f. Path. 1907, I, 214.

[14] We are far from wishing to undertake or to sanction experiments upon human subjects; but it may be of interest to mention in passing that experiments of this nature have been reported repeatedly. We have already referred to the fact (p. 72) that Jenner inoculated the boy, James Phipps, whom he had vaccinated two months before, with virulent smallpox material without producing the disease. The youngster was rewarded for his heroism, in submitting to the experiment, by the gift of a house and garden. In 1798, Jenner furnished further proof that cowpox will protect against smallpox, by vaccinating a child directly from a cowpox vesicle, and then continuing the vaccination from arm to arm through a series of five children, after which all five were variolated, that is to say, inoculated with human smallpox, the result being again negative.

Paraskeva & Zallonis (Gaz. méd. d. Paris, 1872, No. 17; cf. Centrlbl. f. d. med. Wiss., 1872, X, 413) infected a man, aged 55 years, who was hopelessly ill with progressive gangrene, with the purulent content of a pulmonary cavity. After his death, five weeks later, the apex of the right lung was found to contain 17 hard nodules, of lentil size; there were 2 tubercles in the left apex, and 2 of pea-size on the convex surface of the liver. The authors claim that this experiment shows the possibility of transmitting tuberculosis experimentally to human subjects.

In a discussion of the relation of bovine to human tuberculosis, which was published shortly after the British Tuberculosis Congress, Baumgarten (Berl. klin. Woch., 1901, XXXVIII, 894; also Deut. med. Woch., 1901, XXVII, 251, L.) related an experiment upon human subjects which was undertaken almost twenty years before by a physician, since deceased, and was based upon the alleged antagonism between carcinoma and tuberculosis. A number of patients with inoperable, generalized malignant tumors (carcinomas, sarcomas) were infected with virulent bovine tubercle bacilli, by subcutaneous injections of considerable amounts of the cultures, with a view of giving them the advantage that might be possible if Rokitansky's view concerning the antagonism between the two diseases were correct.

vations in human subjects, the stronger becomes the evidence that our clinical observations possess the relation and significance which we are inclined to ascribe to them. In the end, however, the practical clinical results become the sole criterion in the evaluation of any given remedy or therapeutic procedure, entirely regardless of what has been shown in the animal experiment.

In conducting such experimental studies, failures are not necessarily due to the remedy employed, although the efficiency of the latter may vary with the mode of manufacture, with the material from which it is prepared, or even with spontaneous changes occurring with age.

Differences in results obtained by different observers may depend upon a variety of other causes, among which the size of the doses, the frequency and method of administration, the times, modes and degrees of the subsequent infections are of importance. Apparently very slight differences in procedure may become deciding factors and, as their relation may be quite unsuspected for the time being, it may happen that experiments fail repeatedly with the technic of one observer, while they succeed with that of another, even when the same technic has been followed apparently.

On the other hand, the outcome of an experiment may be simulated in one direction or the other, as is well illustrated by our experience with inactivated sera mentioned on p. 94, because we were obliged, for the time, to accept the result even though it was contrary to the one which we had expected. The caution which is implied is recognized by all experienced students in experimental medicine, whereas those with but slight

The patients were neither benefited nor injured by this treatment, as became evident when their bodies were examined after death by Baumgarten.

The personal experiment of Möller is well known, in which this author infected himself with virulent tuberculous material, after having vaccinated himself with his blind-adder tubercle bacilli. Herbert (Tübinger Arbeiten, 1899, III, 207 (216)) established the probable harmlessness of the Rabinowitch butter bacillus, by inoculating himself with a culture which he had obtained. Similar experimental inoculations are fairly frequent in research laboratories.

Finally the vaccinations of children, by Webb & Williams with numbered living, virulent tubercle bacilli, and by Friedmann with living "mitigated" human tubercle bacilli after passage through the turtle organism, must be accepted as experiments upon the human subject, the results of which may not become manifest for many years.

or with no experience are apt to form hasty and premature conclusions. To guard against these it is customary to repeat the experiments regardless of the first results, especially when they appear to contradict those of other observers or if they are not in accord with other facts and experiments, or even with the expectations based upon a well-considered theory which must be the foundation of all original work.

Contradictory results need not be the fault of technic, they may arise from unknown defects of apparatus, reagents, or materials used, or may depend upon the experiment animals themselves. Altogether, experimental studies require not only experience but also much time and patience, whether they be original or only repeat those of others. Caution is called for particularly in the latter case, and studies by an independent investigator are more likely to develop the truth, on a first trial, if the experimenters cooperate and if the same material and technic are employed by both, under the guidance of him whose experiments it is proposed to repeat.

In our earlier studies upon the toxic action of the Vaccine on guinea-pigs, doses from five to fifty milligrams could be given without apparent harm; young, growing animals continued to gain, and full-grown animals showed no loss of weight. Only moderate doses had been used in the experimental treatment of guinea-pigs, upon the results of which we reported in 1912, and no relation seemed to exist between the size of the dose and the time necessary to cause the appearance of specific amboceptors and lysins in the sera of these animals. It then required about ten weeks to produce a maximal amount of specific bacteriolytic amboceptors, regardless of whether the weekly doses were one, five or ten milligrams. It was also found that further treatment did not increase the titer in the complement-fixation test, although it did raise that of the agglutinative power of the serum.

This corresponds with the conclusions of other observers, that there is a time-factor in the acquirement of specific immunity, and also that there is a quantitative limit in the production of bacteriolytic amboceptors, which cannot be increased further once it has been reached. Our observations in the human subject already had suggested the latter point; they dif-

fered from those in the animal experiment in so far as it was possible in the normal human subject to stimulate the complete immunizing response in the course of one week, by means of a single dose of Vaccine.

It still remained to be determined whether or not a single dose, or at least less numerous doses, would be effective, with a proper mode of procedure, in producing the same results in animals. In our endeavor to find an answer to this question, we observed that a complete immunizing response could be elicited, in rabbits and cattle, by the intravenous administration of one or two large doses of vaccine; but even in this case the specific amboceptors required from three to five weeks for their maximal development.

On p. 20 of our report of 1912 we recorded some experiments in a group of immunized and infected animals, giving the results of our autopsy findings in so far as they were available at that time. It now remains for us to give the results of our autopsy findings for the remaining animals of this group, viz., Nos. 9, 12, 14, 15, 16, 17 and 18, which had been treated and infected in the same manner as those whose autopsies we described, but which had been kept for further observation.

GUINEA-PIG No. 9. Weight on day of infection, 620 grams; killed after 496 days; weight 980 grams.

Autopsy: Slightly enlarged, hard cervical lymph glands show fibrous structure; one mesenteric gland, 3 mm., hard; omentum thickened. No other changes. Antiformin residue of smears from cervical and mesenteric glands and omentum negative. *No tuberculosis.*

GUINEA-PIG No. 12. Weight on day of infection, 770 grams; killed after 440 days; weight 1195 grams.

Autopsy: All parts and organs normal, except two fibrous scars in the right lung. Sections negative. *No tuberculosis.*

GUINEA-PIG No. 14. Weight on day of infection, 570 grams; reinfected 712 days after first infection; weight 980 grams. Died 12 days after second infection; weight 680 grams.

Autopsy: First infection site not found; new infection site 3 mm. area of infiltration; omentum and serosa of right testicle contain several doubtful nodules in which no tubercle bacilli are found; peritoneum, liver, spleen show several caseous nodules, 1-2 mm. in diameter, probably pseudotuberculosis of the Pfeiffer type; negative in smears, and no tubercles in sections. No apparent cause for death. *No tuberculosis.*[15]

[15] Control animal for reinfection died of generalized tuberculosis 58 days after infection.

GUINEA-PIG No. 15. Weight on date of infection, 720 grams; died of acute pneumonia 696 days later. Weight before the onset of pneumonia 910 grams; weight at death 610 grams.

Autopsy: All parts and organs normal, except lungs; upper lobe contains a retracted fibrous area 2 by 3 mm. Smear from antiformin residue negative. Left lung and right middle lobe acute pneumonia; smears from antiformin residue negative; sections show no tubercles. *No tuberculosis.*

GUINEA-PIG No. 16. Weight on day of infection, 560 grams; died of acute pneumonia 717 days later. Weight before onset of pneumonia 1000 grams; weight at death 630 grams.

Autopsy: All parts and organs normal, except that the entire right lung is the seat of pneumonic changes. Diplococcus capsulatus in smears from cut surface; no tubercle bacilli in smears of antiformin residue. *No tuberculosis.*

GUINEA-PIG No. 17. Weight on day of infection, 560 grams; died of acute pneumonia 695 days later. Weight before the onset of pneumonia, 1030 grams; weight at death, 650 grams.

Autopsy: All organs and parts normal, except the liver, which shows a fibrous retracted area, 5 mm. in diameter, and the right lung and left upper lobe, which are the seat of pneumonia. Sections show no tubercles. Smears from antiformin residue of liver and lung show no tubercle bacilli. Cultured a small micrococcus from the seared pneumonic portion of the lung. *No tuberculosis.*

GUINEA-PIG No. 18. Weight on day of infection, 530 grams. Reinfected with 0.005 mgr. of tubercle bacilli, subcutaneously, 695 days after the first infection. Weight 880 grams. Killed 121 days after second infection; weight 600 grams.

Autopsy: First infection site not found; second infection site caseous; tubercle bacilli found in smears. Lymph glands regional to second infection site are hard and fibrous. Mesenteric glands hard; bronchial glands, 10 mm., cartilaginous; mesentery contains a few nodules, 1 mm. in diameter, apparently recent. Liver and spleen enlarged, contain several necrotic foci, 2 by 7 mm. in diameter. Right lung, upper lobe, and left lung, lower lobe, in each a firm fibrous mass within which are communicating cavities with caseous contents. Smears show tubercle bacilli; sections from mesentery, liver and spleen show no tubercles; sections from the walls of the lung cavities and from bronchial glands show only fibrous structure; smears from the necrotic foci in liver and also in spleen are negative.[16]

It is difficult to judge correctly of the relation of our reinfections to the lung lesions. It is possible that the fibrous masses, in which the cavities were found, were the result of our first infection. It is also possible, however, that a spontaneous superinfection, prior to our second infection, could have been

[16] Two control animals for reinfection died 24 and 31 days after inoculation; autopsy showed miliary tuberculosis.

responsible, inasmuch as the fibrous walls of pulmonary cavities, which are free from infiltration, must evidently be of long standing, and the time of 121 days from the date of our second infection appears rather short for such changes to occur. The idea that the lung-lesions antedated our second infection might find support from the recorded weight of this animal, which had been 1010 grams six weeks before, and had gradually diminished to 880 grams by the time when our second infection was made; but, inasmuch as the animal had evidently acquired pseudo-tuberculosis of the liver, spleen and mesentery, this can of course have stood in relation to the previous loss.

On the same page we recorded an autopsy upon an immunized animal (Guinea-pig No. 3) in which one tracheal and one bronchial lymph gland was found caseous, and acid-fast granules were found in smears. Guinea-pig No. 43 was infected subcutaneously with caseous material from this tracheal gland, which was followed by suppuration and death on the sixth day. Guinea-pig No. 44, infected subcutaneously with caseous material from the bronchial gland, continued in good health and had doubled its weight at the end of the seventh month, when it was used for another purpose.

In our experiments made since, with a view of substantiating the results reported upon in 1912, we soon met with much greater difficulties, for which we were, at first, as much at a loss to account as we were for some anomalous observations in our bactericidal experiments which we have described already and, in the end, they were found to have an identical cause. After treating the animals with like or larger doses and for like periods of time as before, we often found the specific antibodies to fall much below the titer that we had adopted as our standard, and only traces could be demonstrated in several animals after repeated examinations, while the lytic action of their sera upon tubercle bacilli was less marked, doubtful and not manifest at all. The animals were then treated further, and a new lot was started in which we changed and varied the size of the doses of vaccine. After having given from fourteen to twenty doses, representing totals of vaccine of between 78 and 134 milligrams, we succeeded in 16 of the animals of the first group in obtaining the desired result in serum tests; in a second group we succeeded in 13 animals, after giving from

eleven to twenty-four doses, representing totals of vaccine of between 23 and 145 milligrams. The animals were thereupon infected.

The results of the infections with 0.01 mgr. of tubercle bacilli of human origin, from a growing agar-culture, in emulsion prepared on the day of infection, are given below for both groups.

GROUP I

A. INTRAPERITONEAL INFECTION.

6 PRINCIPALS.

GUINEA-PIG No. 59. Weight 740 grams; died 4 days after infection; weight 700 grams.

Autopsy shows peritoneal exudate which is sterile and free from tubercle bacilli. No apparent cause of death. *No tuberculosis.*

GUINEA-PIG No. 60. Weight 700 grams; died within 24 hours; weight 610 grams.

Autopsy: Abdominal organs congested; slight amount of cellular exudate which shows tubercle bacillus granules with leucocytes; otherwise sterile. No apparent cause for death. *No tuberculosis.*

GUINEA-PIG No. 62. Weight 740 grams; died within 12 hours; weight 700 grams.

Autopsy identical in all respects with that of No. 60. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 63. Weight 710 grams; died after 116 days; weight 670 grams.

Autopsy: Caseous nodule subcutaneously at infection site; no tubercle bacilli found in smears; pseudo lesions of the Pfeiffer type in liver and spleen; smears and sections negative as to tubercle bacilli or tubercles. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 70. Weight 630 grams; died after 10 hours.

Autopsy gave an identical record as that for Nos. 60 and 62. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 130. Weight 770 grams; died after 43 days; weight 580 grams.

Autopsy: Caseous nodule subcutaneously at infection site; no tubercle bacilli found in smears; pseudo lesions of the Pfeiffer type in spleen; smears and sections negative as to tubercle bacilli or tubercles. No cause for death. *No tuberculosis.*

5 CONTROLS.

GUINEA-PIG No. 284. Weight 520 grams; died after 50 days; weight 350 grams.

Autopsy: Caseous nodule subcutaneously and in peritoneum at infection

site; generalized tuberculosis; smears and sections are all positive. Death from *generalized tuberculosis.*

GUINEA-PIG No. 285. Weight 465 grams; died after 56 days; weight 300 grams.

Autopsy record practically identical with that of Guinea-pig No. 284. Death from *generalized tuberculosis.*

GUINEA-PIG No. 286. Weight 485 grams; died after 53 days; weight 350 grams.

Autopsy record practically identical with that of Guinea-pig No. 284. Death from *generalized tuberculosis.*

GUINEA-PIG No. 287. Weight 465 grams; died after 83 days; weight 280 grams.

Autopsy record practically identical with that of Guinea-pig No. 284. Death from *generalized tuberculosis.*

GUINEA-PIG No. 288. Weight 400 grams; died after 37 days; weight 300 grams.

Autopsy: Infection site not found. *Generalized acute miliary tuberculosis.*

B. SUBCUTANEOUS INFECTIONS.

7 PRINCIPALS.

GUINEA-PIG No. 72. Weight 800 grams; died after 148 days; weight 820 grams.

Autopsy: Infection site not found; regional and all other lymph glands normal; 70 cc. of clotted blood in peritoneal cavity, sterile; three hard, fibrous nodules in omentum; hobnail liver (antiformin residue shows no tubercle bacilli; sections show cirrhosis and necrotic areas; no tubercles). Obstruction of portal circulation; point of rupture not found. Death from hemorrhage. *No tuberculosis.*

GUINEA-PIG No. 73. Weight 700 grams; died after 93 days; weight 400 grams.

Autopsy: Infection site caseous; smears show no tubercle bacilli; pseudo lesions in liver; smears and sections negative as to tubercle bacilli and tubercles. No apparent cause for death. *No tuberculosis.*

GUINEA-PIG No. 74. Weight 600 grams; died after 89 days; weight 400 grams.

Autopsy: Infection site caseous, smears show no tubercle bacilli, but many cocci; pseudo lesions in liver and spleen; smears show no tubercle bacilli, but cocci like those found in smears from infection site; sections negative as to tubercles. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 79. Weight 570 grams; died after 146 days; weight 450 grams.

Autopsy: Infection site not found; pseudo lesions in liver; no tubercle bacilli in smears; sections show no tubercles. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 129. Weight 600 grams; died after 48 days; weight 300 grams.

Autopsy: Infection site caseous; smears show no tubercle bacilli; pseudo lesions in liver and spleen; smears and sections negative as to tubercle bacilli and tubercles. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 76. Weight 700 grams; died after 58 days; weight 760 grams.

Autopsy: Infection site not found; regional and other lymph glands normal; abdominal cavity filled with blood; liver very soft and friable; adhesions to diaphragm; spleen contains three 1 mm. hard nodules; smears from antiformin residue from liver and spleen show no tubercle bacilli; no sections made. Death from rupture of abdominal blood vessel. *No tuberculosis.*

GUINEA-PIG No. 135. Weight 600 grams; died after 79 days; weight 490 grams.

Autopsy: Infection site thickened and indurated; smears from antiformin residue show no tubercle bacilli; regional lymph glands hard and fibrous; spleen contains necrotic areas; smears show no tubercle bacilli; liver slightly enlarged, soft, friable; smears from antiformin residue are negative as to tubercle bacilli; sections from liver and spleen show no tubercles. No cause for death. *No tuberculosis.*

2 CONTROLS.

GUINEA-PIG No. 305. Control. Weight 355 grams; died after 16 days; weight 280 grams.

Autopsy shows nothing abnormal. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 362. Control. Weight 420 grams; died after 46 days; weight 320 grams.

Autopsy: Infection site and regional lymph glands caseous; sections from all internal organs positive; tubercle bacilli in smears. *Generalized tuberculosis.*

C. INTRATRACHEAL INFECTIONS.

3 PRINCIPALS.

GUINEA-PIG No. 136. Weight 510 grams; died after 75 days; weight 400 grams.

Autopsy: Infection site not found; regional and other lymph glands normal; pseudo lesions of the Pfeiffer type, in liver and spleen; smears from antiformin residue show no tubercle bacilli; sections are negative as to tubercles. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 137. Weight 630 grams; died after 39 days; weight 510 grams.

Autopsy: Infection site not found; regional and other lymph glands normal; obstruction of large intestine; spleen slightly enlarged; smears from antiformin residue show no tubercle bacilli. Death from intestinal obstruction. *No tuberculosis.*

GUINEA-PIG No. 139. Weight 650 grams; died after 112 days; weight 600 grams.

Autopsy: Infection site not found; cervical and thoracic lymph glands enlarged; omentum thickened, shows numerous minute nodules; large necrotic area in spleen; small necrotic area in liver; all lesions negative in smears and sections, except spleen, in the antiformin residue of which acid-fast rods and granules were found. No apparent cause for death. Pseudo lesions of the Pfeiffer type. The result is doubtful; probably no tuberculosis due to our infection.

2 CONTROLS.

GUINEA-PIG No. 360. Weight 410 grams; died after 42 days; weight 300 grams.

Autopsy: Infection site caseous; regional lymph glands enlarged; numerous miliary tubercles in liver and spleen; abdominal lymph glands enlarged; tubercle bacilli found in smears from antiformin residues from all lesions. *Tuberculosis.*

GUINEA-PIG No. 361. Weight 410 grams; died after 26 days; weight 260 grams.

Autopsy: Infection site and regional lymph glands caseous; tubercle bacilli in smears; sections from all lesions positive. *Generalized tuberculosis.*

GROUP II

Infections the same as in the preceding group, unless stated otherwise.

A. INTRAPERITONEAL INFECTIONS.

4 PRINCIPALS.

GUINEA-PIG No. 134. Weight 700 grams. Infected with 2 mgr. of tubercle bacilli; killed after 161 days; weight 600 grams.

Autopsy: Infection site caseous; tubercle bacilli in smears; pseudo lesions of the Pfeiffer type in liver; otherwise normal (see Autopsy-control No. 1, Guinea-pig No. 714, p. 395).

GUINEA-PIG No. 142. Infected with 2 mgr. of tubercle bacilli; weight 620 grams; killed after 205 days; weight 450 grams.

Autopsy: Infection site not found; regional lymph glands hard and fibrous; fibrous nodules in omentum and lungs; pseudo lesions of the Pfeiffer type in liver and spleen; all smears and sections negative. (See Autopsy-control No. 2, Guinea-pig No. 947, p. 395.) *No tuberculosis.*

GUINEA-PIG No. 154. Weight 680 grams; died of abortion after 248 days; weight 650 grams.

Autopsy: Infection site caseous; no tubercle bacilli found in smears; all organs and parts normal. *No tuberculosis.*

GUINEA-PIG No. 163. Weight 570 grams; killed after 112 days; weight 530 grams.

Autopsy: Infection site and regional lymph glands not found; pseudo lesions of the Pfeiffer type in spleen; smears and sections negative (see Autopsy-control No. 3, Guinea-pig No. 642, p. 395.) *No tuberculosis.*

3 CONTROLS.

GUINEA-PIG No. 505. Control. Weight 400 grams; died after 76 days; weight 420 grams.

Autopsy: Infection site and regional lymph glands caseous; caseous and non-caseous tuberculosis of all organs, tubercles and tubercle bacilli in smears and sections from all lesions; sections from liver and spleen show pseudo lesions. *Generalized tuberculosis.*

GUINEA-PIG No. 517. Control. Weight 370 grams; died after 57 days; weight 300 grams.

Autopsy: Infection site and regional lymph glands caseous; tuberculosis of all organs, tubercle bacilli in smears from all lesions; no sections made. *Generalized tuberculosis.*

GUINEA-PIG No. 516. Control, for Guinea-pigs Nos. 134 and 142. Weight 460 grams. Infected intraperitoneally with 2 mgr. of tubercle bacilli; died after 26 days; weight 340 grams.

Autopsy: Smears and sections from all lesions positive. *Acute miliary tuberculosis.*

B. SUBCUTANEOUS INFECTIONS.

3 PRINCIPALS.

GUINEA-PIG No. 91. Weight 580 grams; killed after 112 days; weight 860 grams.

Autopsy: Infection site caseous; tubercle bacilli in smears; one fibrous nodule in spleen; sections shows no tubercles (see Autopsy-control No. 4, Guinea-pig No. 641, p. 395.) *No tuberculosis.*

GUINEA-PIG No. 138. Weight 700 grams; died after 354 days; weight 980 grams.

Autopsy: All parts and organs normal. No cause for death. *No tuberculosis.*

GUINEA-PIG No. 210. Weight 580 grams; killed after 67 days; weight 740 grams.

Autopsy: All parts and organs normal. *No tuberculosis.*

2 CONTROLS.

GUINEA-PIG No. 504. Control. Weight 560 grams; died after 48 days; weight 370 grams.

Autopsy: Infection site and regional lymph glands caseous; tubercle bacilli in smears; miliary tuberculosis of liver, spleen and omentum; tubercle bacilli in smears. Cause of death: *Miliary tuberculosis.*

GUINEA-PIG No. 518. Control. Weight 545 grams; killed after 68 days; weight 380 grams.

Autopsy: Infection site and regional lymph glands caseous; tubercle bacilli in smears; caseous and non-caseous tuberculosis of all organs; all smears and sections positive. *Generalized tuberculosis.*

C. INTRATRACHEAL INFECTIONS.

6 PRINCIPALS.

GUINEA-PIG No. 150. Weight 880 grams; killed after 76 days; weight 900 grams.

Autopsy: All parts and organs free from tuberculosis; pseudo lesions of the Pfeiffer type in liver and spleen; smears from antiformin residue, and sections are negative. *No tuberculosis.*

GUINEA-PIG No. 151. Weight 610 grams; killed after 161 days; weight 770 grams.

Autopsy: All parts and organs free from tuberculosis; pseudo lesions in liver (see Autopsy-control No. 5, Guinea-pig No. 716, p. 395). *No tuberculosis.*

GUINEA-PIG No. 183. Weight 580 grams; killed after 112 days; weight 600 grams.

Autopsy: All parts and organs normal, except spleen, which shows pseudo lesions of the Pfeiffer type. *No tuberculosis.*

GUINEA-PIG No. 204. Weight 660 grams; killed after 161 days; weight 720 grams.

Autopsy: Infection site not found; regional lymph glands hard and fibrous; one fibrous nodule in spleen; smears from antiformin residue show tubercle bacilli; sections negative as to tubercles. (See Autopsy-control No. 6, Guinea-pig No. 710, p. 395). *No tuberculosis.*

GUINEA-PIG No. 205. Weight 610 grams; killed after 76 days; weight 720 grams.

Autopsy: All organs and parts normal. *No tuberculosis.*

GUINEA-PIG No. 456. Weight 470 grams; killed after 161 days; weight 650 grams.

Autopsy: Infection site open ulcer; smears negative; few fibrous nodules in liver; lung shows an area of livid induration in right apex; smears negative (see Autopsy-control No. 7, Guinea-pig No. 709, p. 395). *No tuberculosis.*

2 CONTROLS.

GUINEA-PIG No. 502. Control. Weight 520 grams; died after 50 days; weight 250 grams.

Autopsy: Infection site not found; regional lymph glands enlarged, caseous; miliary tubercles in all organs; antiformin residue, smears positive from all organs; no sections made. *Acute miliary tuberculosis.*

GUINEA-PIG No. 503. Weight 480 grams; died after 37 days; weight 440 grams.

Autopsy: Infection site not found; regional lymph glands enlarged; otherwise the same record as that for Guinea-pig No. 502. *Acute miliary tuberculosis.*

AUTOPSY CONTROLS

Group No.	G.P. No.	Infected with Saline Emulsion from			Autopsy			Result
		Lesion of	G.P. No.	Weight	Died After Days	Weight	Internal Organs	
1	714	liver	134	470	91	410	Pseudo of liver and spleen	no tbc.
2	947	lung	142	600	119 k.	490	Genuine and pseudo-tuberculosis	tbc.
3	642	spleen	163	460	208	390	Pseudo of liver and lungs	no tbc.
4	641	"	91	430	39	280	" " " " "	" "
5	716	liver	151	360	215 k.	860	Normal	" "
6	710	spleen	204	490	155	500	Pseudo of liver and spleen	" "
7	709	liver	456	490	91	390	" " " " "	" "

It will be noted that death occurred without apparent or sufficient cause in 10 of the 16 principals in Group I, unless pseudo-tuberculosis could be considered in that light; the lesions in the organs appeared to us to be quite insufficient to cause death.

The cause of death in three other animals is evident from the autopsy findings; the deaths in Guinea-pigs 59, 60, 62, which occurred also without apparent cause, are evidently the result of a toxic effect similar to that observed in Nos. 6, 7, 8, 9, 13, 31, 32 and 33 in the bactericidal experiments. The fact that the controls escaped, and that in one case, which was examined bacteriologically, the peritoneal exudate was found sterile in a culture experiment, appears to eliminate bacterial contamination of the tubercle bacillus emulsion which was used for the intraperitoneal infections.

In our results in Group II we have only one death occurring without apparent cause, 354 days after infection. This animal had gained steadily after infection, and weighed 920 grams two weeks prior to its death. It lost 420 grams in the two weeks; and yet all organs were normal on autopsy, except an indurated, livid area of very small extent in the tip of the upper lobe of the right lung, which we have since learned not only to recognize but also to fear as a type of pseudo-tuberculosis which we described elsewhere (p. 318).

Eleven of the principals were killed long after their controls had died. Five of them had pseudo lesions of liver, spleen, or both; two had the same lesions in the lungs as those just mentioned. One died 248 days after infection on account of abortion. All autopsy controls but one were free from tubercu-

losis; the positive case being Guinea-pig No. 947, which had been infected with a saline suspension of a fibrous nodule from the lung of Guinea-pig No. 142.

The animals in this second group behaved better after their infection and, although we found tubercle bacilli in the caseous infection sites of two of them, and in a third one the infection site had ulcerated and was still open, no tubercle bacilli were found and no tuberculous disease was present. More animals had gained in weight since their infection, especially when pseudo lesions were not in evidence. Among our controls we observed a normal course in both groups, excepting possibly one animal, Guinea-pig No. 305 in Group I, which died 16 days after infection, the miliary tubercles present in liver and spleen appearing inadequate to have caused death.[17] The long period of treatment which appeared necessary in the principals was, however, contrary to our experiences in 1911-12, nor were the results as clean-cut as they had been then.

Under the circumstances we were forced to consider the vaccine used in the treatment of these animals; yet to the acceptance of the view that it stood in relation, our observations in the prophylactic and clinical use of the preparation were opposed. the satisfactory results from its employment being demonstrated daily, and fully as good as in the beginning. The only clinical difference that we could find was that the reactions after the injection of the vaccine were not so well marked, and that febrile reactions did not occur as frequently in response to small doses as had been noted at first.

As far as the physical appearance of the Vaccine was concerned, we found no difference on comparing it with a specimen purposely set aside; but a chemical analysis showed that a change had taken place, in that some of the proteins had become reduced, and the missing per cent was shown to be present in the form of nucleic acid. This was a surprise because we had found no such change, or any change whatever in our Watery

[17] The more frequent absence of pseudo-tuberculosis in the control animals appears less remarkable on considering that they had less opportunity for its acquirement by exposure in our animal-houses, because as a rule they were received by us only shortly before they were used, while the principals had been treated for periods of from fourteen to twenty-four weeks before being infected.

Extract of Tubercle Bacilli, after several years, in frequent examinations since we first produced it in our laboratory in 1896. The change in the Vaccine was shown to be actual by repeating the analysis with identical results and, while we continued its study on animals in increasing and variously modified doses and modes of administration, we were obliged, for comparative studies, to await the time when a fresh preparation should be available. We also watched our clinical results as closely as possible with a view of determining the propriety of substituting, temporarily, our Watery Extract of Tubercle Bacilli for the Vaccine; but this was never found necessary or desirable, and we were amply confirmed in this position by spontaneous reports from physicians, which we received with increasing frequency, and also in reply to a special collective inquiry, of highly satisfactory and often brilliant results at the hands of colleagues to whom vaccine had been supplied regularly ever since we ourselves had applied it practically.

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Besides the two groups of experiments in active immunization. which we have considered, a number of other guinea-pigs had been treated, but their serum-tests were, as a rule, unsatisfactory, even after prolonged administration of the Vaccine. In two series, one comprising 24 and the other 38 experiments in which the infections were nevertheless made, very few of the animals or of their controls lived long enough to justify their consideration for the purpose for which they were intended and, with but very few exceptions, all had pseudo lesions when they died. The type of pseudo-tuberculosis appeared to vary with the stock of the breeder. In shipments obtained from different sources, there were animals in which the pseudo lesions were only of the Pfeiffer type; in other shipments the type corresponding with that produced by the Bacillus abortus Bang was found; but the type which is characterized by the indurations in the lungs, mentioned on p. 318, was the most frequent one.

A third series of 62 animals was treated with a PARTIALLY new preparation of vaccine, without any difference whatever being demonstrated in the results; many died during their treatment, only a few of the animals were infected, none of them lived more than five weeks; the controls also died prematurely, the same as the principals, and all animals were found to have

Rabbit No.		Treatment Vaccine		Infection			Autopsy		
Serial	Laborat.	Doses, No.	Total Mgr.	Mode	Bov. T.B. Mgr.	Weight Gm.	Weight Gm.	Infection Site	Reg. Lymph Glands
Infections, Nov. 11, 1912. *Antibodies and Lysis Reach Standard*									
PRINCIPALS									
1	20	4	78	subc.	0.01	1790	2900	0	0
2	21	4	78	"	"	1380	2410	0	0
3	22	20	130	"	"	1380	2650	caseous	fibrous
4	24	14	210	"	"	1900	2100	"	0
5	29	14	620	"	"	1800	2070	0	0
6	31	2	150	"	"	2460	2820	0	0
7	32	1	100	"	"	2000	1800	0	0
CONTROLS									
1	33	—	—	"	"	2140	1300	caseous	caseous
2	34	—	—	"	"	1806	1610	"	"
PRINCIPALS									
8	19	20	70	i. p.	2.0	1906	2150	0	0
9	23	16	130	"	0.01	1900	1470	0	0
10	25	15	240	"	"	1990	3000	0	0
11	26	13	430	"	"	1720	2450	0	0
12	27	15	420	"	"	1800	2200	0	0
13	30	14	620	"	"	1680	1560	0	0
CONTROLS									
3	35	—	—	"	"	1940	1310	caseous	caseous
4	36	—	—	"	"	1820	1680	"	"
Infections, March 13, 1913. *Antibodies and Lysis Reach Standard*									
PRINCIPALS									
14	37	4	200	subc.	0.01	1860	2260	abscess	0
15	42	4	200	"	"	1700	1850	0	0
16	43	4	200	"	"	1850	1980	abscess	caseous
17	45	4	200	"	"	1800	1000	0	0
18	46	4	200	i. p.	"	2270	1900	0	0
19	47a	4	200	"	"	2200	2470	0	0
20	48	4	200	"	"	2470	2450	0	0
21	49a	4	200	"	2.0	1910	1420	0	0
22	50	3	30	"	"	900	2500	0	0
23	51	3	30	"	"	1000	1700	0	0
CONTROLS									
5	73	—	—	subc.	0.01	1140	900	caseous	caseous
6	74	—	—	"	"	1210	850	"	"
7	75	—	—	i. p.	"	1080	900	"	"
8	76	—	—	"	"	1250	1070	"	enlarged
9	136	—	—	"	2.0	2450	2280	"	"
10	137	—	—	"	"	800	750	0	"
Infections, Nov. 6, 1913. *Antibodies and Lysis Slightly below Standard*									
PRINCIPALS									
24	99	10	76	subc.	0.01	2060	2360	abscess	0
25	104	13	90	"	"	2300	2600	0	0
26	171	11	45	"	"	1630	2330	scar	0
27	95	12	91	i. p.	"	1830	2570	"	0
28	97	12	122	"	"	1870	1450	0	0
29	100	14	152	"	"	2600	2500	caseous	0

Autopsy						Result	
Internal Organs	Pseudo Lesions	Cause of Death	After Days	Smears	Sections		
Normal	0	killed	546	—	—	no tbc.	
"	0	"	280	—	—	" "	
"	0	"	295	t.b. inf. site	—	" "	
Omentum thickened; spleen slightly enlarged	0	no cause	183	0	0	" "	
9 hard nodules, 1–2 mm. in liver	liver	killed	233	0	0	" "	
Normal	0	coccidiosis	229	0	0	" "	
"	0	pneumonia	42	0	0	" "	
Generalised tbc.	0	tbc.	69	posit.	posit.	tbc.	
" "	0	"	82	"	"	"	
Soft nodule in right broad ligament; 2 yellow hard nodules in periton.; scar in liver	0	killed	148	t.b. perit. & br. lig.	—	no tbc.	Autopsy Control ( ) G.P. #515
Normal	0	no cause	220	0	—	" "	
Adhesions omentum to liver	0	killed	546	0	0	" "	
3 nodules, 2 mm. in liver; 2 in omentum	liver	pneumonia	247	t.b. omt.	0	" "	
Normal	0	killed	161	—	—	" "	
"	0	trauma sepsis.	56	—	—	" "	
Generalised tuberculosis	0	tbc.	104	posit.	posit.	tbc.	
" "	0	"	95	"	"	"	
Lung few doubtful nodules	lung ?	no cause	74	t.b. inf. site	0	no tbc.	
Normal	0	pneumonia	68	0	0	" "	
Lung, 2 grayish-yellow foci	lung	coccidiosis	69	t.b. inf. site	0	" "	
Liver, 6 hard nodules, 2 mm.	Liver	trauma sepsis.	107	0	0	" "	Autopsy Control ( ) G.P. #635
Normal	0	degen. liver & spleen	38	0	0	" "	Autopsy Control ( ) G.P. #567
"	0	trauma sepsis.	99	0	0	" "	
"	0	pneumonia	68	0	0	" "	
Omentum, 3 nodules, 1 mm.	0	no cause	16	t.b. omt.	0	" "	
Normal	0	killed	187	0	0	" "	
Spleen slightly enlarged	0	"	115	0	0		
Generalised tbc.	0	tbc.	72	posit.	posit.	tbc.	
" "	0	"	102	"	"	"	
" "	0	"	69	"	"	"	
" "	0	"	84	"	"	"	
" "	0	"	51	"	"	"	
Generalised acute miliary	0	"	17	"	"	"	
Large intest. 5 mm. cas. encapsul. nodule; left lung, 6 fibrous foci, 1–2 mm.	0	killed	96	t.b. and gran. in lung	posit.	no tbc.	
Each lung few fibrous nodules, 1–3 mm.	0	"	133	0	0	" "	
Normal	0	"	133	0	0	" "	
Oment. 2 calcar. nodules; liver, several fibr. nod.; lungs, few hard nodules in each	0	"	70	t.b. and gran. in lung and liver	0	" "	
Normal	liver	no cause	22	0	0	" "	
"	0	killed	77	t.b. inf. site	0	" "	Coccidiosis of liver

Rabbit No.		Treatment Vaccine		Infection			Autopsy		
Serial	Laborat.	Doses, No.	Total Mgr.	Mode	Bov. T.B. Mgr.	Weight	Weight	Infect. Site	Reg. Lymph Glands
30	105	13	113	i. p.	0.01	2500	2710	0	enlarg. fibr.
31	238	7	50	"	"	1770	2080	0	" "
32	239	8	55	"	0.05	1420	1660	0	0
33	241	7	50	"	0.01	1600	1850	0	0
34	242	7	50	"	"	1670	2240	0	0
CONTROLS									
11	279	—	—	subc.	0.01	2330	1780	caseous	caseous
12	280	—	—	"	"	2280	1530	"	"
13	285	—	—	"	"	1700	1500	"	"
14	284	—	—	i. p.	"	1750	1650	"	"
15	286	—	—	"	"	2280	1980	"	"
16	287	—	—	"	"	1900	1660	"	"
Infections, Dec. 11, 1913. *Antibodies and Lysis Slightly below Standard*									
PRINCIPALS									
35	255	4	50	subc.	0.05	2000	2500	abscess	0
36	268	3	30	"	"	1980	2240	scar	0
37	160	19	111	"	0.01	1960	2330	caseous	0
38	185	20	98	"	"	2160	2480	abscess	0
39	228	3	145	"	1.0	2300	2900	caseous	caseo
40	217	3	30	i. p.	0.05	1980	2080	scar	0
41	247	6	30	"	"	2080	2650	0	0
42	265	3	30	"	"	2080	2460	scar	0
43	156	19	100	"	0.1	1830	2300	0	0
44	159	19	100	"	"	2450	2800	0	0
45	210	19	98	"	"	2160	2580	scar	0
46	214	3	155	"	"	2450	2730	0	0
47	273	3	60	"	"	1910	2900	0	0
48	274	3	60	"	"	1720	1570	0	0
49	184	20	107	"	1.0	2080	1830	caseous	enlarged
50	204	18	121	"	1.0	2400	1450	fibrosis	0
51	209	17	100	"	1.0	1980	1580	"	0
Infections, Dec. 11, 1913. *Antibodies and Lysis much below Standard*									
PRINCIPALS									
52	220	15	145	i. p.	0.05	2380	1720	0	fibrocas.
53	240	7	50	subc.	0.01	1630	1950	caseous	caseous
54	249	7	50	i. p.	0.05	2100	2500	caseous	0
55	250	7	50	"	"	1920	1880	fibrcas.	0
56	251	7	50	"	"	1750	1980	scar	0
57	252	7	50	"	"	1785	2170	caseous	0
58	253	2	30	"	"	2000	1800	fibrcas.	caseous
59	254	3	40	"	"	2130	1800	caseous	"
60	256	5	60	"	"	2000	2500	scar	0
61	263	3	30	"	"	1500	2170	0	0

Autopsy						Result	
Internal Organs	Pseudo Lesions	Cause of Death	After Days	Smears	Sections		
Lungs, fibrous areas in each	liver	killed	96	0	0	no tbc.	
Lungs, 3 fibrous nodules, 3 mm.	0	"	70	0	0	" "	
Lungs, 2 fibr. scars; liver, 1 yellow nodule	0	chr. colitis	71	0	0	" "	
Lungs, many yellow areas. 1–5 mm.	lungs	killed	118	0	0	" "	
Lungs, numerous fibr. areas, 3–7 mm.	"	"	96	0	0	" "	Coccidiosis abdm.cyst.
Generalised tbc.	0	tbc.	122	posit.	posit.	tbc.	
" "	0	"	133	"	"	"	
Miliary tubercles in liver, spleen, lung	0	"	58	"	"	"	
" " " " " "	0	"	57	"	"	"	
Miliaries in omentum, liver, spleen; few in lungs	0	pneumonia	30	"	"	"	
Generalised acute miliary.....	0	tbc.	48	"	"	"	
One fibrous nodule in each lung	0	killed	97	t.b. inf. site	0	no tbc.	
Lungs, numerous nodules, 2–4 mm.	lung	"	97	0	0	" "	
Left lung, 6 nodules, 1–3 mm.	"	"	97	0	0	" "	
Lungs, 11 hard nodules	"	"	99	t.b. inf. site	0	" "	
Omentum thickened. Numerous fibrous areas in lungs	liver and lung	no cause	94	0	0	" "	
Omentum a fibrous mass; lungs, 3 fibr. nod.	0	killed	86	0	0	" "	
Lung, rt. apex fibrous; 4 hard nodules in left lung	0	"	97	0	0	" "	
Lung, rt. apex completely fibrous; 3 hard nodules in cortex of left kidney	0	"	97	0	0	" "	
Normal	liver	"	87	0	0	" "	
6 hard nodules in rt. lung; 5 nodules in left	lung	"	97	0	0	" "	
Normal	0	"	100	0	0	" "	
4 hard nodules in rt. lung; 3 in left	lung	"	87	0	0	" "	
1 hard nodule in left lung	0	"	100	0	0	" "	
Several nodules in liver and spleen	liver, spleen	trauma	79	0	0	" "	
Numerous non-caseous hard nodules in liver, lung, peritoneum	liver, lung	killed	87	t.b. inf. site	mes. gld. posit.	lymph gld. tbc.	
Numerous nodules in liver, spleen	" spleen	no cause	37	0	0	no tbc.	
Numerous hard nodules 1 mm. in mesentery, liver, spleen, lungs	lung	" "	82	t.b. inf. site, liver mesent. lung	spleen fibrous tubercle	+ bc	
Few fibr. nodules in kidneys, testes, intestines; lungs adhesions, numerous yellow foci, 2–3 mm.	0	no cause	74	t.b. intest.	lung posit.	tbc.	
Few nod. in liver, spleen; many caseous foci in lungs	liver, spleen lung	tbc.?	87	posit.	posit.	"	
Rt. lung, 4 hard nodules 2 mm.	0	killed	97	t.b. inf. site	lung fibr. tubercle	no tbc.	
Fibroid areas, 1–7 mm. in liver, lungs	liver, lung	"	97	" "	0	0	
Few hard nodules in periton., mesent., omentum, several soft yellow nod. in liver, spleen	liver and spleen	"	97	" "	oment.	posit. fib. lesion	
Few soft nod. in oment.; few hard nodules in lungs	liver, lung	"	97	0	0	no tbc.	
Few recent tubercles in liver, spleen; few 1–5 mm. soft nod. in diaphragm, pericard.	0	"	83	posit.	posit.	slight tbc.	
Numerous tubercles in omentum, mesentery; liver, many; spleen, one 2 mm. soft nod.	liver, spleen	"	97	"	not made	tbc.	
Omentum, mass of fibrous nodules; periton. few; intest., few; diaphr., few fibr. nod.	0	"	97	0	"	no tbc.	
Necrotic areas in liver. Lungs, rt. lower, few hard nodules; left, pneumonia	lung, liver	pneumonia	75	0	0	"	

Rabbit No.		Treatment Vaccine		Infection			Autopsy		
Serial	Laborat.	Doses, No.	Total Mgr.	Mode	Bov. T.B. Mgr.	Weight	Weight	Infect. Site	Reg. Lymph Glands
62	266	3	30	i. p.	0.05	1970	1520	scar	caseous
63	267	3	30	"	"	2400	2600	scar	
64	164	19	117	"	0.1	2250	1820	caseous	enlarged
65	187	20	97	"	"	1950	1630	"	0
66	189	20	109	"	"	2130	2450	scar	enlarged
67	203	18	128	"	"	2400	2800	0	0
68	208	17	76	"	"	1850	1600	scar	0
69	211	17	128	"	"	2260	1530	caseous	caseous
70	216	15	145	"	"	1870	2080	0	0
71	218	16	155	"	"	2000	1520	caseous	caseous
72	219	18	145	"	"	2480	1430	fibrocas.	enlarged
73	222	15	125	"	"	2680	2100	caseous	0
74	248	7	50	"	"	2000	2590	"	0
75	270	3	60	"	"	1740	2200	scar	0
76	271	3	60	"	"	1605	1860	caseous	0
77	272	3	60	"	"	2010	1780	scar	0
78	276	3	60	"	"	1730	2200	caseous	0
79	277	3	60	"	"	1900	1680	"	0
CONTROLS									
17	261	—	—	subc.	0.01	1100	900	caseous	caseous
18	279	—	—	"	"	2330	1780	"	enlarged
20	294	—	—	"	"	2080	2080	"	0
21	297	—	—	"	"	1820	2060	"	0
22	306	—	—	"	0.05	1930	2280	"	enlarged
23	307	—	—	"	"	1360	1680	"	caseous
24	308	—	—	"	"	1800	1700	"	enlarged
25	293	—	—	"	0.1	2010	1800	open ulcer	"
26	295	—	—	"	"	2600	1900	caseous	caseous
27	298	—	—	"	"	2000	1580	"	"
28	289	—	—	"	1.0	1670	1550	"	"
29	302	—	—	i. p.	0.05	1800	1300	"	"
30	303	—	—	"	"	1730	1580	"	"
31	304	—	—	"	"	930	700	"	"
32	305	—	—	"	"	1230	1100	"	"
33	309	—	—	"	"	1310	1120	"	"
34	311	—	—	"	"	1800	1480	"	"
35	296	—	—	"	0.1	1030	900	"	"
36	299	—	—	"	"	1780	1400	fibrocas.	"
37	300	—	—	"	"	1270	880	"	"
38	301	—	—	"	"	1230	700	"	"

Autopsy						Result	
Internal Organs	Pseudo Lesions	Cause of Death	After Days	Smears	Sections		
Omentum, many 3 mm. caseating nodules; mesent. and diaphr., few hard nod.; lungs, many fibrous foci, 3–10 mm.	0	killed	97	posit.	posit.	tbc.	
Lungs numerous hard yellow foci	lungs	"	97	0	0	no tbc.	
Several fibr. nod. in mesent., oment., intest.; many hard yellow foci, 2–3 mm. in lungs	"	"	99	0	posit.	fibroid lesions	
Lungs, many hard yellow foci; mesent. and oment., few; intest., one hard nodule	"	"	87	posit.	"	fibroid lesions	
Intestine, 3 fibrous nodules; diaphr., few; lungs, numerous 1–3 mm. soft nod.; testes caseous	0	"	86	0	" diaphr. lung	tbc.	
Diaphr., few 1 mm. nod. on both surfaces; lungs, numerous fibrous areas, 2–7 mm.; spleen, 8 soft nodules	lung	"	97	t.b. spleen	spleen, posit.	slight tbc.	
Oment. rolled up, fibrous mass includes pancreas; intestine, spleen, kidneys, numerous 1–2 mm. nodules; lungs, many 1–2 mm. soft yellow nodules	"	"	99	t.b. in spleen intest.	oment. posit.	"	
Omentum and mesentery recent tubercles; liver few yellow nodules; lungs, many 1–3 mm. nodules	lung, liver,	pseudo-tbc.	84	—	posit. oment. mesent.	"	
Lung, 6 nodules, 5 mm. in right; 5 nodules, 3 mm. in left	" "	killed	100	0	0	no tbc.	Coccidiosis of liver
Lungs, several fibrocas. nod. in each; kidneys, 8 hard nod. 1 mm. upon periton. surface	lung	no cause	78	t.b. inf. site	lung posit.	slight tbc.	
Recent tubercles in periton.; few nod., 1 mm., spleen, kidney; caseating tubercles in tunica of testes. Many large and small fibrocaseous areas in lungs	"	tbc.	53	—	—	"	
Kidneys and spleen, several 1 mm. nodules; lungs, many 1–3 mm. hard yellow nod.	"	trauma sepsis.	60	t.b. inf. site	—	no tbc.	
Intestine, 1 fibrocaseous nodule; rt. lung, 1 nod. 2 mm.; left lung, 2 nod. 2 mm.	0	killed	97	0	0	" "	
Tunica of left testis, 10 hard nod.; rt. lung, 8; left lung, 6 fibrous nodules	0	"	100	0	0	" "	
Many non-caseous hard nodules in liver, spleen, lungs; oment., thickened	liver, spleen lung	"	100	t.b. inf. site	oment. posit.	tbc.	
Normal	0	exudative peritonitis	46	0	0	no tbc.	
Tunica of left testis, 6 hard. nod. 1 mm.	testicle	trauma sepsis.	65	t.b. inf. site.	0	" "	
Mesentery, oment. and intest., numerous nod., 1–2 mm.; lung, many yellow foci.	lung	killed	100	" "	—	" "	
Generalized tbc.	0	tbc.	92	posit.	posit.	tbc.	
" "	0	"	122	"	"	"	
" "	0	"	100	"	"	"	
" "	0	killed	100	"	"	"	
Extensive caseating tbc. of lungs; slight in abdom. organs	0	"	97	"	"	"	
Generalized tbc.	0	"	97	"	"	"	
" "	0	"	97	"	"	"	
" "	0	coccidiosis	55	"	"	"	
" "	0	tbc.	100	"	"	"	
" "	0	killed	100	"	"	"	
" "	0	tbc.	51	"	"	"	
" "	0	"	54	"	"	"	
" "	0	"	84	"	"	"	
" " pulm. cavity	0	"	75	"	"	"	
" "	0	"	85	"	"	"	
" "	0	"	96	"	"	"	
" "	0	"	99	"	"	"	
" "	liver	"	60	"	—	"	
" " (incl. tuberculous ulceration of bladder)	0	"	53	"	posit.	"	
Generalized tbc.	0	"	62	"	—	"	
" "	0	"	78	"	—	"	

pseudo-tuberculosis. Realizing that it would be a loss of time and money to go any further, we killed and autopsied all guinea-pigs which we had on hand, regardless of whether they were being treated or had been used for experiments, or whether they belonged to our supposedly normal stock. This course appeared fully justified when we found that, among 118 of supposedly normal guinea-pigs which had never been used for any experiments, there were only two which were free from evidences of pseudo- or of genuine tuberculous infection.

Including this lot of 118 animals, nearly 1000 autopsies were made during the early months of 1914, after which we did not undertake other than bactericidal experiments because, one of us having agreed to be in London in May or June of the year, there was no time to conduct or finish experiments of active immunization. We succeeded in obtaining only one lot of guinea-pigs which were free from pseudo-tuberculosis, and the bactericidal experiments made with this lot were fully satisfactory in their results.

ACTIVE IMMUNIZATION OF RABBITS

We have not met with the same degree of difficulties when we used rabbits as experimental animals. We found much fewer cases of spontaneous genuine and pseudo infections, but coccidiosis has caused us numerous losses, and pneumonias have at times been troublesome. The rabbits which had pseudo-tuberculosis bore their treatment with vaccine better than did the guinea-pigs and, on the whole, we had fewer deaths from incidental diseases, and very few in which the cause of death was left obscure.

The results of several experiments which we made in order to determine the bactericidal action of human and bovine sera after vaccination, upon virulent tubercle bacilli of bovine origin, for rabbits, are added to Table I.

The accompanying tabulation will, we believe, support us sufficiently in our contention that rabbits can be immunized the same as other animals. In some of the animals a vaccine made from the bovine type of tubercle bacilli, in others one made from the human type, was used for treatment; but there was no

apparent difference in the antibody-contents of their sera or in the results after infection.

While we have not infected any large animals heretofore. we treated twelve calves with vaccine, giving single large doses of ten cubic centimeters intravenously to some, and two such doses to others. We contented ourselves so far with the demonstration of specific antibodies and lysins which were found to have developed, the same as they were observed in smaller laboratory animals, and they were demonstrable quantitatively in like amounts, the maximal titer being reached from four to five weeks after a single dose or two doses had been administered. We have reexamined the sera of these calves at various periods after vaccination and, up to two and one-half years later, we have not failed to find these antibodies, although quantitatively they appeared to have diminished; whereas they were never found in sera from calves or grown cattle not previously treated, or in their sera when these were used for controls. Infections were not made in these animals because they are part of the valuable stock belonging to the dairy of the Winyah Sanatorium. The sera of four of the vaccinated calves were, however, used successfully for bactericidal experiments with tubercle bacilli of human origin.

CHAPTER XIII

THERAPEUTIC EXPERIMENTS

Experimental therapeutic studies upon animals have been undertaken by numerous observers, and were attempted also in our laboratory, especially since the introduction of tuberculin in 1890. Our attempts to demonstrate the therapeutic value of the various tuberculins and of other products of the tubercle bacillus failed, or at least we did not meet with even a fair degree of uniformity in our results; we gave up further studies in this direction in 1901, after the therapeutic employment of the Watery Extract of Tubercle Bacilli in animals had yielded contradictory results in which the failures far exceeded the apparent successes. In some of the successes, however, the restriction of tuberculous changes to the infection site and to regional lymph glands, or the unmistakable reparative processes found in the involved organs, suggested that our treatment had exerted a favorable influence upon the clinical course of the disease, and we should have been in a position to accept and to claim a relation of these positive results to the treatment, had it not been for the fact that the majority of the animals, treated in exactly the same manner, not only failed to present like evidences of a favorable influence, but that we found instances in which the progress of the disease was rapidly fatal, sometimes more rapidly than was the case in untreated controls. We concluded at that time either that the guinea-pig was not a suitable animal for experiments of this kind or that our preparation was incapable of counteracting an artificial infection uniformly. In the course of our more recent researches we were induced, however, to make a therapeutic trial in a certain lot of animals which could not be used for any other purpose, on account of spontaneously acquired tuberculosis, and we consider our observations in these animals to be of sufficient interest and importance to be recorded, although our results were really not much better than were those which

we had observed with the use of the Watery Extract many years ago. The difference lies in the fact that we now understand the reasons for the failures, and that we can interpret the results more correctly.

The original number of this lot of guinea-pigs was four hundred. Eight of them were found dead in the shipping-boxes on arrival, and were not autopsied; 3 died before they had received any vaccine; 18 died after the first dose; 31 after the second dose; and 14 after the third dose of vaccine, a total of 74 animals.

Of the 66 animals which came to autopsy, after receiving from one to three doses of vaccine, none showed lesions which could be considered sufficient, in number or extent, to have caused death; on the contrary, in most instances the lesions were comparatively slight. In some, pseudo-tuberculosis was found either alone or associated with genuine tuberculosis; in others, nothing abnormal was found beyond small, circumscribed areas of intense congestion, which we accepted as evidences of focal reactions to the vaccine, because of the subsequent findings in smears and sections. A characteristic feature appeared to be the rapid loss of weight in all these animals. Our autopsy findings were as follows:

Macroscopic lesions of genuine tuberculosis	46
Macroscopic lesions of genuine and pseudo-tuberculosis	10
Focal reactions only	8
Focal reactions, and lesions of pseudo-tuberculosis	2

Focal reactions were observed in all animals that had genuine tuberculous lesions.

With these results before us, it was of course impossible to be certain whether all unsuitable animals had been weeded out, and it was decided to let the animals rest for a week or ten days without treatment, and to watch their behavior. No further deaths occurred during this time; most of the animals had lost decidedly in weight during their treatment, and the loss continued in some, others remained stationary, and still others showed more or less gain.

The remaining 326 animals, together with 16 supposedly normal controls which had not been treated previously, were then infected subcutaneously in the right axilla with 0.2 cc. of an

emulsion of tubercle bacilli of human origin, equal to 0.01 milligram. This dose, given subcutaneously, had been found to be virulent in preceding control-experiments, having caused death from tuberculosis in guinea-pigs after an average of 66 days.

Ten of the previously treated animals were now set aside in order to serve for "treated" controls; leaving 316 principals for further treatment; our observations were made therefore on 316 principals, 10 treated controls which had received three doses of vaccine each, and 16 untreated controls. Of the 316 principals we lack autopsies for six, the animals having been lost. In order to show the subsequent behavior to infection and treatment, we give the autopsy results of the remaining 310 principals and the 26 controls, as their deaths occurred after infection, and before and subsequently to each dose of vaccine. The descriptions of the lesions refer to those found in internal organs; these lesions were controlled by the examination of smears and sections, and frequently by positive culture-experiments in cases of pseudo lesions.

15 principals and 5 treated controls died, 1 to 3 days after infection, and before further treatment was given.

PRINCIPALS:

Macroscopic lesions; old genuine tuberculosis	9
Macroscopic lesions; old genuine and pseudo-tuberculosis	2
Focal reactions only	2
Pseudo-tuberculosis only	1
Pseudo-tuberculosis and pneumonia	1
The infection site was not found	13
The infection site was congested	2

The regional lymph glands were normal in all.

CONTROLS.

Macroscopic lesions; old genuine tuberculosis	3
Macroscopic lesions; old genuine and pseudo lesions	1
Focal reactions only	1
The infection site was not found	3
The infection site was hemorrhagic	1
The infection site was congested	1

The regional lymph glands were normal in all.

Focal reactions were noted in all animals with genuine tuberculosis.

After the first dose of vaccine: 46 principals and no controls died between 5 and 10 days after our infection.

Macroscopic lesions; old genuine tuberculosis only	36
Macroscopic lesions; old genuine and pseudo-tuberculosis	3
Focal reactions only	5
Pseudo-tuberculosis only	1
Pseudo-tuberculosis and peritonitis	1
The infection site was not found	31
The infection site was congested	14
The infection site was hemorrhagic	1
The regional lymph glands were enlarged, 2-3 mm.	35
The regional lymph glands were not enlarged	11

Focal reactions were noted in all animals with genuine lesions.

After the second dose of vaccine: 24 principals and no controls died between 11 and 17 days after our infection.

Macroscopic lesions; old genuine tuberculosis	14
Macroscopic lesions; old genuine and pseudo-tuberculosis	3
Focal reactions only	3
Pseudo-lesions only	3
Apparently normal animals[1]	1
The infection site was not found	13
The infection site was congested	9
The infection site was caseous	2
The regional lymph glands were not enlarged	1
The regional lymph glands were enlarged	21
The regional lymph glands were caseous	2

Focal reactions were noted in all animals with genuine lesions.

After the third dose of vaccine: 23 principals and 3 controls died between 18 and 23 days after our infection.

PRINCIPALS:

Recent genuine lesions	8
Recent and old genuine lesions	6
Old genuine lesions only	4
Old genuine and pseudo lesions	3
Pseudo lesions only	2

[1] Smears from antiformin residue and sections of the apparently normal spleen showed tubercle bacilli and tubercles; infection site and regional lymph glands were normal.

The infection site was not found..... 6
The infection site was congested..... 10
The infection site was caseous..... 7

The regional lymph glands were not enlarged..... 3
The regional lymph glands were enlarged..... 16
The regional lymph glands were caseous..... 4

Focal reactions were observed in 18 of the 21 animals with genuine lesions.

CONTROLS:

No. 1 not treated. Infection site caseous; regional lymph glands caseous; pseudo-tuberculosis of lung.

No. 2 treated. Infection site caseous; regional lymph glands caseous; genuine old lesions and pseudo lesions.

No. 3 treated. Infection site congested; regional lymph glands enlarged. No apparent cause for death.

After the fourth dose of vaccine: 10 principals and 1 control died between 27 and 31 days after our infection.

PRINCIPALS:

Recent genuine and pseudo lesions..... 1
Old genuine lesions..... 3
Old genuine and pseudo lesions..... 5
Pseudo lesions only..... 1

The infection site was caseous..... 10

Regional lymph glands caseous..... 9
Regional lymph glands enlarged..... 1

Focal reaction in 1 animal with old genuine lesions.

CONTROLS:

1 treated control; old and recent genuine lesions; infection site caseous; regional lymph glands caseous.

After the fifth dose of vaccine: 8 principals and 2 controls died between 32 and 39 days after our infection.

PRINCIPALS:

Genuine recent lesions only..... 2
Genuine recent and pseudo lesions..... 2
Genuine recent and old lesions..... 2
Genuine old and pseudo lesions..... 1
Pseudo lesions only..... 1

Infection site caseous in..... 8

Regional lymph glands caseous in..... 8

No focal reactions were noted.

CONTROLS:

1 untreated control. Genuine recent and pseudo lesions; infection site caseous; regional lymph glands caseous. No apparent cause for death.

1 treated control; infection site caseous; regional lymph glands caseous. No apparent cause for death.

After the sixth dose of vaccine: 17 principals and 2 controls died between 40 and 45 days after our infection.

PRINCIPALS:

Genuine, only recent lesions found	7
Genuine, old and recent, and pseudo lesions	2
Genuine, old and recent, no pseudo lesions	7
Pseudo lesions only	1
Infection site not found	7
Infection site caseous	10
Regional lymph glands not found	1
Regional lymph glands enlarged, soft	2
Regional lymph glands enlarged, hard	3
Regional lymph glands caseous	11

No focal reactions were noted.

CONTROLS:

1 treated control. Infection site caseous; regional lymph glands fibrous; old and recent genuine lesions.

1 untreated control. Infection site caseous; regional lymph glands caseous; genuine generalized tuberculosis and pseudo lesions in liver and spleen.

After the seventh dose of vaccine: 41 principals and 2 controls died between 45 and 52 days after our infection.[2]

PRINCIPALS:

Caseous and pseudo lesions	2
Genuine non-caseous and caseous lesions	5
Genuine non-caseous lesions only	17
Genuine non-caseous and pseudo lesions	11
Genuine fibrous lesions only	2
Genuine fibrous and pseudo lesions	1
Lymph gland tbc. only; internal organs free except pseudo	3
Infection site, open ulcer	4
Infection site caseous	34

[2] It was impossible to differentiate between old and recent tuberculous lesions in these and the subsequent autopsies.

Infection site caseous encapsulated..... 1
Infection site fibrous..... 1
Infection site not found..... 1

Regional lymph glands caseous..... 16
Regional lymph glands fibrocaseous..... 13
Regional lymph glands fibrous..... 9
Regional lymph glands not enlarged..... 3

No focal reactions were noted.

CONTROLS: 2 untreated controls.

No. 1. Infection site caseous; regional lymph glands caseous; genuine generalized miliary and caseous lesions.

No. 2. Infection site caseous; regional lymph glands caseous; genuine miliary and caseous tuberculosis; pseudo lesions in liver.

After the eighth dose of vaccine: 39 principals and 3 untreated controls died between 52 and 66 days after our infection.

PRINCIPALS:

Genuine caseous and non-caseous lesions..... 4
Genuine caseous and fibrous lesions..... 2
Genuine non-caseous only..... 6
Genuine non-caseous and pseudo lesions..... 8
Genuine non-caseous and fibrous lesions..... 12
Genuine non-caseous and fibrous and pseudo lesions..... 4
Genuine fibrous lesions only..... 1
Genuine fibrous and pseudo lesions..... 1
Lymph gland tbc. only; intern. organs free except pseudo..... 1

Infection site, open ulcer..... 6
Infection site, caseous..... 24
Infection site, caseous encapsulated..... 6
Infection site, healed scar..... 2
Infection site, fibrous thickening..... 1

Regional lymph glands caseous..... 8
Regional lymph glands fibrocaseous..... 13
Regional lymph glands fibrous..... 18

No focal reactions were noted.

CONTROLS: 3 untreated controls.

No. 1. Infection site caseous; regional lymph glands caseous; non-caseous and pseudo lesions; lived 63 days. Miliary tuberculosis.

No. 2. Infection site caseous; regional lymph glands caseous; caseous and non-caseous lesions; lived 63 days. Generalized tuberculosis.

No. 3. Infection site caseous; regional lymph glands caseous; non-caseous lesions. Lived 66 days. Generalized tuberculosis.

After the ninth dose of vaccine: 35 principals and 5 untreated controls died between 66 and 75 days after our infection.

PRINCIPALS:

Genuine caseous and non-caseous lesions	1
Genuine caseous and fibrous lesions	4
Genuine caseous, fibrous and pseudo lesions	2
Genuine non-caseous and pseudo lesions	2
Genuine non-caseous and fibrous lesions	4
Genuine non-caseous, fibrous and pseudo lesions	4
Genuine fibrous lesions only	3
Genuine fibrous and pseudo lesions	7
Reg. lymph gland tbc. only; internal organs free	5
Reg. lymph gland tbc. only; internal organs pseudo	1
No tuberculosis; no pseudo	2
Infection site, open ulcer	8
Infection site, caseous	16
Infection site, caseous encapsulated	6
Infection site, encapsulated fibrous thickening	2
Infection site, scar	1
Infection site, not found	2
Regional lymph glands caseous	2
Regional lymph glands fibrocaseous	8
Regional lymph glands fibrous	24
Regional lymph glands not found	1

No focal reactions were noted.

CONTROLS (5 untreated):

Infection site caseous	5
Regional lymph glands caseous	5
Non-caseous and caseous lesions; no pseudo	3
Non-caseous and caseous lesions; pseudo	1
Non-caseous lesions only miliary	1

After the tenth dose of vaccine: 15 principals and no controls died between 75 and 81 days after our infection.

PRINCIPALS:

Genuine caseous and fibrous lesions	3
Genuine fibrous lesions only	2
Genuine fibrous and pseudo lesions	4
Genuine non-caseous and fibrous lesions	2
Lymph gland tbc. only; internal organs free	4
Infection site, open ulcer	1
Infection site, caseous	5

Infection site, caseous encapsulated..... 6
Infection site, scar..... 2
Infection site, not found..... 1

Regional lymph glands fibrocaseous..... 2
Regional lymph glands fibrous..... 13

No focal reactions were noted.

After the eleventh dose of vaccine: 15 principals and the last 3 untreated controls died between 81 and 91 days after our infection.

PRINCIPALS:

Genuine fibrous, caseous and non-caseous lesions..... 5
Genuine non-caseous lesions only..... 2
Genuine fibrous lesions only..... 1
Genuine fibrous and pseudo lesions..... 5
Regional lymph gland tuberculosis only..... 1
No genuine lesions; only pseudo lesions..... 1

Infection site, open ulcer..... 5
Infection site, caseous..... 3
Infection site, caseous encapsulated..... 4
Infection site, not found..... 1

Regional lymph glands caseous..... 2
Regional lymph glands fibrocaseous..... 5
Regional lymph glands fibrous..... 7
Regional lymph glands not found..... 1

No focal reactions were noted.

CONTROLS:

Nos. 1 and 2. Infection sites caseous; regional lymph glands caseous; genuine caseous and non-caseous lesions in both. Generalized tbc.

No. 3. Infection site, open ulcer; regional lymph glands caseous; caseous and non-caseous lesions. Generalized tbc.

After the twelfth dose of vaccine: 5 principals died between 91 and 97 days after our infection.

PRINCIPALS:

Genuine non-caseous and pseudo lesions..... 2
Genuine fibrous lesions only..... 2
No genuine, only pseudo lesions..... 1

Infection site, open ulcer..... 3
Infection site, caseous..... 1
Infection site, fibrous scar..... 1

Regional lymph glands fibrocaseous..... 2
Regional lymph glands fibrous..... 3

No focal reactions were noted.

CONTROLS are all dead.

After the thirteenth dose of vaccine: the last 18 principals were killed 100 days after our infection.

PRINCIPALS:

Genuine non-caseous and fibrous lesions..... 4
Genuine fibrous lesions only..... 7
No genuine lesions..... 2
No genuine lesions, only pseudo..... 2
Regional lymph gland tbc. only..... 3

Infection site, open ulcer..... 2
Infection site, caseous..... 3
Infection site, scar..... 4
Infection site, not found..... 9

Regional lymph glands fibrocaseous..... 3
Regional lymph glands fibrous..... 13
Regional lymph glands not found..... 2

No focal reactions were noted.

RÉSUMÉ

In this series 326 animals are to be considered, an unknown number of which had acquired a spontaneous infection with tubercle bacilli, and all of which had received three doses of vaccine before our infections were made. Of the 326 animals, 10 were set aside for controls; 316 were treated further, 6 of them were lost, leaving 310 to be accounted for. Sixteen supposedly normal guinea-pigs were added, to serve as untreated controls.

In 20 of the animals the relation of our infections to death, which followed in the course of 1 to 3 days, is of peculiar interest in that 18 showed focal reactions in their tuberculous lesions, apparently as a result of the superinfection, similarly as we observed them in animals dead of toxemia after the administration of the vaccine. The relation of the superinfection is supported by the fact that no deaths had occurred for nearly two

weeks prior to infection, and further by our observations that the number of deaths always increased promptly after a new dose of vaccine was administered. Another interesting feature in connection with these deaths was that animals which had only pseudo lesions appeared to have been subject to a like unfavorable influence from the action of the vaccine, and finally that, in the large majority of the principals and also in some of the controls, the extent of disease found was comparatively slight and that, judging from our former experiences with infections of previously normal animals, the macroscopic appearance of the lesions found was rarely sufficient to justify the assumption that death was due directly to the lesions found in these treated animals.

With only few exceptions, both smears and sections were made of the lesions found at autopsy and the classification of the lesions is based upon the result of their examination.

The fatal effects observed upon tuberculous animals, from the subcutaneous administration of one to three milligrams of our vaccine (whereas 500 mgr. of old tuberculin are necessary to produce similar results), serve to illustrate the difference of action of potency between preparations made from culture-media and those which contain the body-extractives of the tubercle bacillus.

Focal reactions were noted in genuine lesions up to 18 days after infection; then they became less frequent, and none were found later than the 31st day.

The first evidences of fibrosis made their appearance 40 days after infection, including one treated control; they appeared first in regional lymph glands, then they became manifest in the infection sites, and also in internal organs.

Commencing with the 66th day after infection, which corresponds with the average lethal effect of the culture in the dose administered, the infection sites began to be obliterated; the regional lymph glands were not always found; caseous lymph gland-tuberculosis then came to be noted only occasionally; the principals no longer showed active progressive lesions in their organs.

Of 168 treated animals which died or were killed between 45 and 100 days after infection, there were 35 in which the genuine lesions of internal organs, found at autopsy, were fibrous;

17 whose internal organs were free from genuine tubercle; and 8 without lesions, the animals having entirely resisted or overcome our infections. The last 12 controls which came to autopsy during this period all had died of genuine tuberculosis. It would, therefore, seem that a favorable influence can justly be attributed to the treatment in at least 60 instances, that is, in 35.7 per cent of the animals which lived 45 days or longer, or were killed when all our pending experiments were terminated. In a number of other animals, in which caseation and recent tubercles were not found, a tendency to arrestment seemed to exist.

We are of the opinion that better results would have been secured had we omitted our own infections and contented ourselves with dividing the animals into two groups, treating one-half of them with vaccine, and keeping an equal number without treatment, for comparison, since it had become evident that the great majority of these animals had actually incurred a genuine infection spontaneously before our infections were made.

We also erred in giving too large doses of vaccine, especially at the beginning of the treatment, and lost in consequence a large number of the tuberculous animals from the effects of focal reactions and consequent toxemia. As soon as circumstances are favorable, it is our intention to undertake further therapeutic experiments. It appears that the degree of success depends to a considerable extent upon the proper mode of procedure, which must be learned by experience in like manner as in the treatment of human tuberculosis.

In order to illustrate the primary localization of spontaneous infections in guinea-pigs, as it was observed by us in our autopsies, we append the details of a few of the protocols.

Primary tuberculosis of the lungs. Guinea-pig No. 961. Received Oct. 4, 1913; died Oct. 10, 1913. Shows numerous nodules, ½ to 1 mm. in diameter, throughout both lungs. Nothing else abnormal, especially no enlargement of superficial lymph glands or of those in chest or abdomen. Smears from antiformin residue of lung tissue show many acid-fast rods; in sections, typical tubercles with beginning caseation are found. No apparent cause of death.

Primary tuberculosis of lung. Guinea-pig No. 951. Received Sept. 29, 1913; was given 2 mgr. of vaccine subcutaneously Oct. 1; died Oct. 5, 1913. Autopsy: no apparent cause of death; site of injection of vaccine

not found; abdominal contents apparently normal; lungs, right upper lobe deeply congested areas; no enlarged lymph glands; smears of antiformin residue of lung tissue show acid-fast rods; sections show typical tubercles.

Primary tuberculosis of lung. Focal reaction. Guinea-pig No. 953. Received Sept. 29, 1913; was given 2 mgr. of vaccine subcutaneously Oct. 1; died Oct. 6, 1913. Autopsy shows no cause for death. Abdominal organs appear normal; upper portions of both lungs contain many fibrocaseous areas, 2-5 mm. in size, which are surrounded by zones of intense congestion. Bronchial glands enlarged, not caseous. Smears from lung lesions and bronchial glands contain acid-fast rods; sections taken from lung tissue contain miliary and caseating tubercles.

Primary tuberculosis of lung. Focal reaction. Pseudo-tuberculosis of liver and spleen. Guinea-pig No. 986. Received Sept. 29, 1913; was given two doses of vaccine, each 3 mgr., subcutaneously, Oct. 1 and 8; died Oct. 15, 1913. Loss of weight 90 grams. Inguinal glands are enlarged, soft; mesenteric glands enlarged, 6 mm., hard. The liver contains several necrotic areas, 2-5 mm.; spleen enlarged. Apex of right lung is full of caseating areas with peripheral hyperemia. Smears from liver, mesenteric glands, inguinal glands and spleen are negative as to acid-fast rods; those from lung contain such rods. Sections taken from liver and spleen contain no tubercles; those from lungs contain breaking-down tubercles.

Primary tuberculosis of lungs. Guinea-pig No. 1139. Received Sept. 29, 1913. Weight on Oct. 1, 1913, is 520 grams. Treated with three doses of vaccine, 1 mgr. each, subcutaneously, between Oct. 1 and 15. Infected Oct. 27, 1913, with 0.01 mgr. of virulent tubercle bacilli, subcutaneously in right axilla. Weight 400 grams. After infection received two further doses of vaccine, 1 mgr. each. Died Nov. 10, 1913; weight 250 grams.

Autopsy shows no apparent cause for death. Infection site not found; regional lymph glands enlarged, 3 mm.; mesenteric glands enlarged, soft. No macroscopical lesions in abdominal organs. Lungs, a small patch of focal reaction in each apex. Smears: axillary glands and lungs, acid-fast rods; mesenteric glands negative. Sections taken from mesenteric glands, hyperplasia; axillary glands, round-cell infiltration; no tubercles. Lung: recent tubercles.

Primary tuberculosis of lung. Guinea-pig No. 1195. Received Oct. 4, 1913; weight on Oct. 9, 500 grams. Treated with two doses of vaccine, 1 mgr. each, subcutaneously. On Oct. 27, weight 480 grams. Infected with 0.01 mgr. of virulent tubercle bacilli, subcutaneously in right axilla; then treated with three doses of vaccine. Died Nov. 18, 1913; weight 330 grams.

Autopsy shows no apparent cause for death. Infection site congested; regional lymph glands enlarged, soft; inguinal glands enlarged, 5 mm., hard; spleen 15 by 25 mm.; lungs, dense tuberculous infiltration in both apices; lower lobes congested. Smears: liver, mesenteric glands, negative; infection site, right axillary glands and lungs, acid-fast rods. Sections taken from liver, doubtful tubercles and fibrosis; from mesenteric glands and lungs, typical tubercles.

Primary abdominal tuberculosis; extension to lung; with focal reactions. Guinea-pig No. 952. Received Sept. 29, 1913. Was given 1 mgr. of vaccine subcutaneously Oct. 1, 1913; died Oct. 5, 1913. Autopsy: no apparent cause of death. Omentum thickened, many nodules intensely congested. Spleen enlarged, many nodules, 1 mm.; mesenteric glands enlarged, 5 mm.; lungs show small circumscribed areas of intense congestion; thoracic glands not enlarged. Smears of antiformin residues from omentum, spleen, mesenteric glands, lungs, show acid-fast rods; sections taken from lung show recent tubercles; from omentum, spleen and mesenteric glands, caseating and recent tubercles.

Primary abdominal tuberculosis. Guinea-pig No. 1055. Received Oct. 4, 1913. Was given three doses of vaccine, subcutaneously, 2 mgr. each, up to Oct. 10; infected Oct. 27, 1913, subcutaneously, with 0.01 mgr. of virulent tubercle bacilli. Weight on day of infection, 320 grams; died Oct. 29, 1913; weight 230 grams.

Autopsy: No apparent cause of death. Infection site congested; regional lymph glands slightly enlarged; mesenteric glands enlarged, 3 mm.; the mesentery and a loop of intestine congested. Spleen shows many miliary nodules; all other organs and contents of thoracic cavity are normal in appearance. Smears from spleen and mesenteric gland show acid-fast rods; sections taken from spleen contain typical tubercles.

Primary tuberculosis of inguinal glands. Pseudo-tuberculosis of liver and lung. Guinea-pig No. 1136. Received Sept. 29, 1913. Was given three doses of vaccine subcutaneously, 3 mgr. each, between Oct. 1 and 15, 1913. Infected Oct. 27, 1913 subcutaneously, in right axilla, with 0.01 mgr. of virulent tubercle bacilli. Weight 410 grams. Received one further dose of vaccine, 3 mgr., Oct. 30. Died Nov. 2, 1913. Weight 360 grams.

Autopsy shows no cause of death. Infection site congested; regional lymph glands enlarged, 3 mm., soft; inguinal glands congested, 3 mm. The peritoneal cavity contains one cubic centimeter of bloody serum; adhesions between diaphragm and liver; mesenteric glands enlarged, 3-4 mm., soft. The liver contains two nodules, 2-3 mm., apparently caseous. Lungs contain several small areas of pneumonic infiltration; no enlarged thoracic glands are found. Smears from liver and mesenteric glands are negative; those from inguinal glands contain acid-fast rods. Sections taken from lung show peribronchial and perivascular infiltration; from liver, fibrosis with necrotic center, no tubercles; from axillary glands, round-cell infiltration, no tubercles; from inguinal glands, typical tubercles, some of them caseating.

Primary abdominal tuberculosis. Pseudo-tuberculosis of liver. Guinea-pig No. 975. Received Sept. 29, 1913; died Oct. 13, 1913. No apparent cause of death. Inguinal glands enlarged, 2 mm. Peritoneum congested; omentum thickened; liver, few hard nodules on surface; spleen enlarged; mesenteric glands enlarged, 3 mm.; left lung, pneumonic area in base; thoracic glands normal. Smears of antiformin residues from spleen, mesenteric and inguinal glands, and pneumonic lung tissue show acid-fast rods; liver negative. Sections taken from spleen and pneumonic lung contain

typical tubercles; liver negative as to tubercles. It appears that this animal acquired a later, independent infection of the left lung.

The following autopsy illustrates the results of our own infection in a guinea-pig which had pseudo-tuberculosis of the liver and spleen:

Guinea-pig No. 1651. Received Sept. 29, 1913. Weight 600 grams. Treated with three doses of vaccine, 2 mgr. each, subcutaneously, from Oct. 1 to 15, 1913. Weight 430 grams. Infected Oct. 27, 1913, with 0.01 mgr. of virulent tubercle bacilli, subcutaneously in the right axilla. Weight 500 grams. After infection, treated with four doses of vaccine; died Nov. 25, 1913. Weight 330 grams.

Autopsy shows infection site 10 mm., caseous; regional lymph glands 10 mm., caseous; mesenteric glands, 15 mm., soft; spleen and liver full of miliary nodules; all other organs apparently normal. Smears from infection site and right axillary glands show acid-fast rods; mesenteric glands, liver and spleen negative. In sections taken from liver, spleen and mesenteric glands no tubercles are found.

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Although we lost, or found to be unsuitable, a large number of guinea-pigs in the course of our experimentations, we have persisted in these studies in order to discover the related cause of the difficulties which we encountered after having had uniform success for over two years. The results which we have again observed under the difficulties which existed, and described, appear to us sufficiently numerous and controlled to show that our early observations can be duplicated in normal animals with a suitable specific vaccine; likewise that, in case of failure, the cause need not lie in the lack of efficiency on the part of the vaccine. The relation of pseudo-tuberculosis to the failures was strikingly apparent in the animals used for treatment in the laboratory of Sir A. E. Wright, in London and many of them died during treatment, or their sera failed to comply with our standard in complement-fixation and in bacteriolytic tests; but for the good fortune of finding a limited number of normal animals for the bactericidal experiments with sera taken after vaccination, the results would have been left sufficiently in doubt to require a repetition of the work.

We consider the destruction of virulence of tubercle bacilli by subjecting them to the action of an active immune serum *in vitro*, taken after the administration of a single dose of vaccine, as the nearest approach to a human experiment and as of deciding value in so far as animal experiment can replace it, or as it may support the results of clinical observations.

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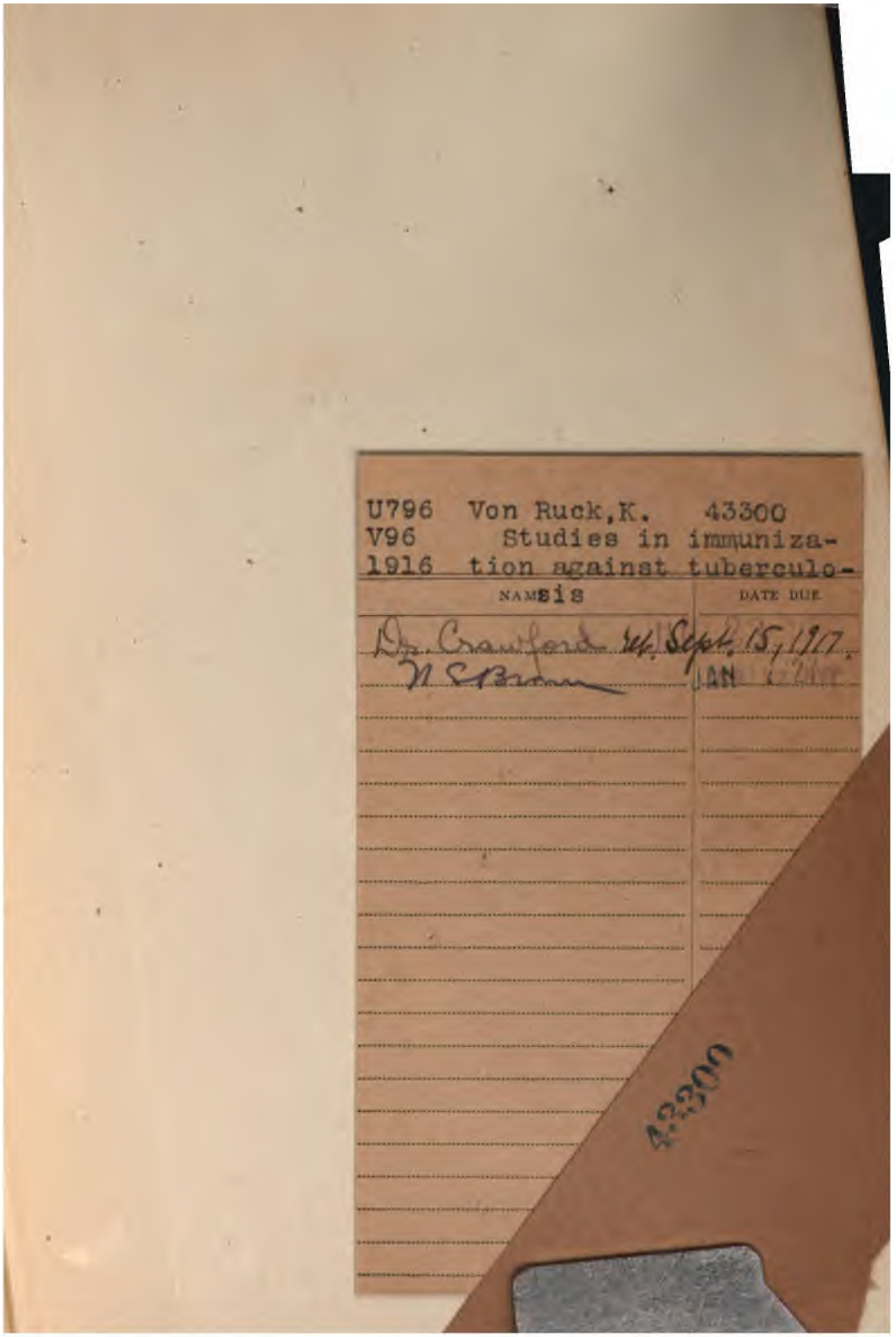
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